Preface

The National Center for Toxicological Research (NCTR) is the primary, agency-wide laboratory research center of the Department of Health and Human Services' (HHS) U.S. Food and Drug Administration (FDA). NCTRis co-located with the Office of Regulatory Affairs' (ORA) Arkansas Regional Laboratory (ARL) on FDA's Jefferson Laboratories Campus in Jefferson, Arkansas, approximately 30 miles south of Little Rock.

NCTR, in partnership with researchers from government, academia, and industry, provides innovative research to develop, refine, and apply current and emerging technologies to improve and facilitate safety evaluations of FDA-regulated products. NCTR fosters national and international research collaborations and communications to promote rapid exchange of theories and emerging sciences with promise for improving the quality and effectiveness of regulatory decisions. NCTR also supports the national and international training of scientists from around the world in the practices of modern toxicology to propagate the principles of regulatory science supporting product-safety evaluation and efficacy.

NCTR's science implements the objectives outlined in FDA's Strategic Goal 2.1 *(Advance Regulatory Science and Innovation),* FDA's Strategic Goal 3.1 *(Advance Food Safety and Nutrition)*, and FDA's Strategic Goal 3.2 *(Promote Public Health by Advancing the Safety and Effectiveness of Medical Products)*. Parallel to the Strategic Goal Program, NCTR's efforts also follow the FDA Critical Path objective for selecting, developing, and evaluating preclinical safety evaluations that can most easily be translated to practices at the clinical level.

NCTR's staff provides customized safety assessments of chemicals and materials of FDA interest. The comprehensive assessments identify information gaps and apply research solutions to obtain data required for FDA to make strategic and scientifically based safety decisions. These projects involve coordinating expertise in several disciplines, including: general biochemical and molecular markers of safety and toxicity, neurotoxicology, microbiology, chemistry, genetic and molecular toxicology, and systems-biology assessments to characterize biomarkers for an individual's susceptibility to toxicants, disease risk, and health status. Ongoing work in the multidiscipline, multi-institutional studies evaluating bisphenol A (BPA) and nanosilver exemplify this approach.

Although studies like the BPA example are designed to identify and resolve specific data gaps required for regulatory decisions, the studies are further designed to evaluate the general biology and physiology principles governing the specific biological responses. In studying these more general relationships, NCTR projects provide the basis for identifying general strategies for evaluation of similar toxicants, and identifying potential biomarkers of toxicity and safety that may provide proven paths to regulatory

decisions in the future.

NCTR also has invested in several emerging technologies for their potential applications in both increasing the accuracy of safety evaluations and reducing the amount of time required for scientific decisions. The newer disciplines include: noninvasive imaging using Magnetic Resonance Imaging (MRI) Spectroscopy (MRS), Positron Emission Technology (PET), Computed Tomography (CT) for monitoring biochemical function in live animals; array technologies, including genomics and proteomics capable of measuring hundreds, if not thousands, of gene and protein changes simultaneously; and development and qualification of bioinformatic sciences to collect, integrate, and evaluate data.

NCTR has formalized bioinformatics and biostatistical efforts into a new Division of Bioinformatics and Biostatistics to enhance FDA's capacity to collect, analyze, and visualize the unprecedented abundance of biological data resulting from the omics revolution. NCTR also has developed and staffed specialized core laboratories with the Office of Regulatory Affairs to characterize nanoscale materials used in safety evaluations, as well as completing facilities for inhalation experiments of toxicants.

Emerging technologies are deployed in parallel with ongoing traditional study designs to provide real-time comparisons of results to accepted technologies, and to maximize identification of potential biomarkers of toxicity to be used in further translational research studies and/or in supporting clinical evaluations of safety and treatment. This synergistic strategy is used to shorten the time to discovery, to qualification, and acceptance of new science in the safety evaluation or regulated products and postmarket evaluations in clinical use.

A significant contribution to our research program also is the investment in sharing knowledge gained during the development, execution, and reporting of the results from cutting-edge studies. NCTR has aggressively developed collaborations and consortia with scientific staff in all disciplines from other FDA Centers, as well as in other government agencies, academia, and industry. One example of this effort is the use of ArrayTrack™™, a software tool developed at NCTR to store, analyze, and interpret DNA microarray data. ArrayTrack™™ is being used by FDA Product Centers in assessing pharmacogenomic and other omics data submitted by the regulated industry. Publication of these studies in peer-reviewed journals has provided the basis for standards and approaches for the routine use of genomic data in the safety-assessment paradigm.

Increasing levels of imports of regulated products and cultural exchange has provided U.S. public health with a global challenge. FDA is engaging this issue in several programs, and NCTR has expanded its efforts in communications to engage scientists from emerging economies. NCTR formed a Global Regulatory Summit as a forum to discuss emerging sciences safety evaluations, exchange perspectives on regulatory programs,

and provide a forum to exchange training opportunities. Cooperation and communication, as well as advancing the principles of regulatory science through training and scientific exchange are key factors to enhanced domestic and global health.

/s/

William Slikker, Jr., Ph.D.

Director, NCTR

NCTR Overview

Vision

The National Center for Toxicological Research (NCTR) is an FDA research center that provides global leadership and innovative scientific solutions in support of FDA's mission to improve public health.

Mission

NCTR conducts scientific research and develops innovative tools and approaches for FDA to protect and promote public health.

NCTR:

- Provides innovative and interdisciplinary toxicology research that anticipates future needs and promotes personal and public health.
- Develops novel translational research approaches to provide FDA/HHS with sound scientific infrastructure and multidisciplinary scientific expertise targeted towards addressing critical Agency, Department, and public-health needs.
- Engages with scientists across FDA and other government agencies, industry, and academia in cooperative efforts to strengthen the scientific foundations vital to developing sound regulatory policy and leverages resources in order to promote the international standardization and global harmonization of regulatory science.
- Participates in/or leads national and international consortia for the development of harmonized standards for technologies and risk-assessment methods vital to FDA's regulatory and public-health mission.
- Provides and encourages multidisciplinary training and fosters national and international collaborations with scientists from government, academia, and industry.

Strategic Plan

NCTR's Strategic Plan sets forth our long-term strategic goals and objectives. The plan also details specific actions we are committed to taking as we carry out our mission to provide global leadership and innovative scientific solutions in support of FDA's mission to improve public health. This Strategic Plan charts NCTR's course for the future, focusing on three strategic goals. The three strategic goals NCTR established to accomplish its mission include:

Goal 1: Advance scientific approaches and tools necessary to support public health.

Goal 2: Develop new and innovative outreach communications materials, methods, and processes that inform and engage NCTR's internal and external target audiences.

Goal 3: Modernize administrative management to support FDA/HHS science goals.

The NCTR Strategic Plan can be found on the FDA website at: www.fda.gov/NCTRStrategicPlan.

Research Structure – FY 2011 (through May 2012)

Established by executive order in 1971, NCTR is internationally recognized for the conduct of scientific research that supports the FDA mission to bring safe and efficacious products to market and reduce the use of adverse health effects.

The research divisions include interdisciplinary teams of scientific experts that conduct fundamental and innovative laboratory research that translates knowledge and technology into processes that improve the safety assessment of FDA-regulated products and reduces the risk of adverse effects from products on the market. NCTR science is structured into divisions having specific disciplines that work as cross-functional teams on projects in three research programs: 1) Personalized Nutrition and Medicine, 2) Strengthen Surveillance and Risk Analysis, and 3) Enhancing Medical Product Safety.

Through May 2012, NCTR research divisions included:

- Division of Biochemical Toxicology
- Division of Genetic and Molecular Toxicology
- Division of Microbiology
- Division of Neurotoxicology
- Division of Personalized Nutrition and Medicine
- Division of Systems Biology

NCTR Organizational Structure Changes

NCTR recently realigned its organizational structure to achieve several important objectives:

- streamline organization function
- consolidate management role to provide more efficient services
- realign scientific expertise to provide critical mass in delivering regulatory-research capabilities to FDA.

Note: *The information provided in this report reflects the organizational structure in effect prior to the reorganization in June, 2012.*

New Research Structure – Effective June 2012

Effective June 2012, NCTR management reorganized its science staff into the following divisions that work as cross-functional teams on NCTR research projects.

After May 2012, NCTR research divisions included:

- Division of Biochemical Toxicology
- Division of Bioinformatics and Biostatistics
- Division of Genetic and Molecular Toxicology
- Division of Microbiology
- Division of Neurotoxicology
- Division of Systems Biology

Division of Systems Biology Changes

The division is no longer organized into six Centers of Excellence. It is now made up of three branches:

- Biomarkers and Alternative Models Branch
- Innovative Safety and Technologies Branch
- Personalized Medicine Branch

The research conducted in the Center of Excellence for Bioinformatics formerly within the Division of Systems Biology was moved into the new Division of Bioinformatics and Biostatistics. The remaining five Centers of Excellence listed below were merged into the three newly formed branches within the Division of Systems Biology.

- Center of Excellence for Functional Genomics
- Center of Excellence for Hepatotoxicity
- Center of Excellence for Innovative Technologies
- Center of Excellence for Metabolomics
- Center of Excellence for Proteomics

Division of Bioinformatics and Biostatistics

This division was created with the goal of enhancing FDA's capacity to collect, analyze, and visualize the unprecedented abundance of biological data resulting from the omics revolution.

Division of Personalized Nutrition and Medicine

The biostatistics research conducted in this division was moved to the new Division of Bioinformatics and Biostatistics. The remaining research conducted in the former division was moved under the new Personalized Medicine Branch within the Division of Systems Biology.

Science Advisory Board

Function

The Science Advisory Board (SAB) advises the NCTR Director in establishing, implementing, and evaluating the scientific research programs conducted at NCTR. NCTR conducts innovative scientific research that assists the FDA Commissioner in fulfilling FDA's regulatory responsibilities. Through site-visit reviews and annual meetings, NCTR's SAB provides an extra-agency scientific program review of the research programs at NCTR. The recommendations of the SAB are critical to the scientific rigor of the studies conducted at NCTR. Members of the SAB and the SAB Chair are selected by the FDA Commissioner, or designee, from among leading authorities in fields related to toxicological research.

FY 2011 Accomplishments

The NCTR SAB held a subcommittee site visit on August 16 and 17, 2011, to perform an in-depth review of the NCTR/ORA Nanotechnology Core Facility (NanoCore). This subcommittee review was different from most subcommittee reviews, which focus on the accomplishments of NCTR Research Divisions. This subcommittee review was conducted to provide early advice for the NanoCore's direction. Key components of the review included: 1) a determination of the leading FDA needs in the area of nanotechnology, 2) an assessment of the NanoCore's organization, 3) an understanding of how projects are developed within the NanoCore, 4) a review of the current facility and staffing plan, and 5) an understanding of ongoing and proposed research projects. While the subcommittee heard presentations on individual studies, the feedback on those studies was given at the meeting and was not included in the report.

The subcommittee report noted that the NanoCore is comprised of excellent facilities, equipment, and a well-qualified staff. As organized today, the NanoCore is available for use by NCTR investigators as needed for their individual research projects. To meet the needs of the agency, the main recommendation of the subcommittee is to change the facility from a Core Facility into a Center of Excellence with a clear mission statement and research strategy that centers on regulatory nanoscience. The subcommittee report was presented and accepted at the full NCTR SAB meeting.

The annual full meeting of the NCTR SAB was held November 8 and 9, 2011. The meeting started with the NCTR Center Director providing an update on activities across the Center, NCTR's strategy for communication and training – designed to extend the reach and impact of NCTR's research and NCTR's collaborations. As a measure of success, the Director discussed the number of scientific publications generated by NCTR researchers during the past six years. In 2011, the number of publications will be similar

to or slightly more than the 163 scientific publications generated in 2010. NCTR's 40th anniversary celebration, which included a Global Summit on Regulatory Science and Innovation, a reception and dinner at the Governor's Mansion, and an event at the Center.

The Global Summit on Regulatory Science and Innovation, hosted by FDA's Office of International Programs (OIP) and NCTR, explored the future of research as a tool for advancing regulatory science, food safety, medical technologies, and public health through a series of presentations and panel discussions. The event was well-attended by international scientists, academics from several universities in Arkansas, NCTR and other FDA scientists, state and local government leaders, and private industry leaders. The Global Summit was followed by a gala dinner at the Arkansas Governor's Mansion to celebrate the successes of NCTR and promote a higher profile for regulatory research in the future. The following day there was an anniversary event at NCTR, which included speeches by a number of elected officials from Arkansas and the signing of an historic Memorandum of Understanding between FDA and the State of Arkansas to set up a virtual Center of Excellence for Regulatory Science. The partnership includes the University of Arkansas System, Arkansas State University, FDA/NCTR, and the State of Arkansas, and will work to forward regulatory science, especially in the area of nanotechnology. The Director closed with a slide summarizing the NCTR budget for the last five years and noted that this year's budget is likely to be similar to that of last year and will be supplemented by monies received through collaborations with other groups.

The Acting Deputy Director of the Office of Counterterrorism and Emerging Threats provided an overview of FDA's Medical Countermeasures Initiative (MCMi) that was launched in 2010. The MCMi, which aims to streamline and enable MCM development and regulatory evaluation, is based on three pillars: 1) Enhance the Review Process, 2) Advance MCM Regulatory Science, and 3) Optimize Legal, Regulatory, and Policy Approaches.

The chair of the Division of Neurotoxicology Subcommittee Site-Visit Review team outlined the key recommendations of that report. The subcommittee visited NCTR on May 26-27, 2010. The Director of the Division of Neurotoxicology presented a detailed response to the seven strategic recommendations listed in the Subcommittee Site-Visit Review Team report and also responded to some of the project-specific recommendations.

On November 9, 2011, the NCTR Center Director presented a talk on the challenges and opportunities for research to advance regulatory science. Recent studies have investigated the reasons why products fail to reach the market. Using pharmaceutical products as an example, it is clear that the inability to recognize safety issues is a major cause of product failure both before and after marketing. The disconnect between the investment in basic science research and the development of products to improve health lead to the recognition that there needs to be an increased emphasis on

regulatory science. Regulatory science is the science of developing new tools, standards, and approaches to assess the safety, efficacy, quality, and performance of FDA-regulated products. NCTR, with it mission to 1) provide innovative and interdisciplinary toxicology research, 2) develop novel translational research approaches to provide FDA/HHS with sound scientific infrastructure and multidisciplinary scientific expertise, 3) engage with scientists within and outside the U.S. government to promote the international standardization and global harmonization of regulatory science, 4) participate in/or lead national and international consortia for the development of harmonized standards for technologies and risk-assessment methods, and 5) provide multidisciplinary training and foster national/international collaborations with scientists from government, academia, and industry; is uniquely positioned to support regulatory science.

Meeting attendees also heard updates from each of the NCTR research divisions regarding their programs, their individual accomplishments, and their future research plans. The SAB attendees also heard presentations from the other government representatives at the meeting, including FDA colleagues. These presentations focused on the upcoming regulatory challenges faced by their respective organizations and the potential role of research collaborations to meet those challenges.

SAB Membership Roster

CHAIR:

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NCTR Advances Research through Outreach and Collaboration

NCTR has actively sought and participated in collaborative, cooperative partnerships with other scientific and regulatory organizations, especially where the partnership augments the mission of NCTR and FDA through the use of NCTR's unique resources. These opportunities to leverage resources, both public and private, enable NCTR to address questions of common concern to both FDA and the collaborating agency. These partnerships have led to substantial research advances that have resulted in significant improvements in long-term public health, such as regulatory guidance, mechanistic understanding, and advanced methodology.

Interagency Agreements

An Interagency Agreement (IAG) is a formal financial partnership with another government agency. NCTR has been fortunate in establishing IAGs with other government agencies to conduct research on problems of common interest to FDA and the collaborating agency. The most significant, in terms of size, is the IAG between NCTR/FDA and the National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP).

National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP)

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FDA has had an IAG with NIEHS since 1992. NIEHS/NTP conducts toxicology studies at the request of federal agencies, including FDA. The IAG is an instrument that allows toxicology studies on chemicals or substances nominated to the NTP to be studied using the unique resources and facilities at NCTR. This research, conducted under the IAG, provides FDA the ability to better assess study design and initial data on the safety of FDA-regulated products, thereby providing FDA and other regulatory agencies with data from studies optimized for risk assessment.

The 1992 agreement provided support for five FDA-priority chemical/agent NTP nominations. The success of the studies and program led to an expansion that allowed continued collaborative toxicity testing on compounds of interest to FDA and NTP. The IAG has led to the investigation of the mechanism-of-action and toxicity assessment of many classes of chemicals, including food contaminants, cosmetics, endocrine-disruptor compounds, food cooking by-products, dietary supplements, drugs, and anesthetics. In response to experimental-design needs for compounds studied under the IAG, the IAG supported the development of the Phototoxicity Research and Testing Laboratory (NTP

Center for Phototoxicology) and the NCTR/ORA Nanotechnology Core Facility.

All toxicology studies conducted under the IAG are designed with input from FDA regulatory scientists, NCTR and NIEHS scientists, scientists from other federal agencies, and invited subject-matter experts. The IAG utilizes resources from public funds and exceptional scientific expertise to provide the best possible assessment of product safety through toxicological studies.

The IAG fulfills one of the strategic goals of NCTR (Strategic Goal 1, Advance Scientific Approaches and Tools Necessary to Support Public Health) through the conduct of toxicology studies that will provide FDA with appropriate data for quantitative risk assessment of compounds. In addition, the studies are accompanied with mechanism-of-action and biomarker studies that allow scientific understanding of the toxicology process and provide information for translation of the safety assessment to humans.

The Office of Scientific Coordination is responsible for generating the background documents for many of the FDA-nominated chemical substances to NTP. The documents are complete literature reviews of the use, pharmacokinetics, human exposure, and toxicity of the nominated substance. The Office of Scientific Coordination wrote four nomination documents in FY 2011 and will continue to produce these review documents to support FDA's need for assistance from NTP in understanding the risk of chemical substances to human health.

Toxicological studies on numerous compounds have been supported since 1992. Many of the compounds are listed below with the nominating Center in parenthesis.

- Acrylamide (CFSAN)
- α and β hydroxy acids dermal (CFSAN)
- AIDS therapeutics (Zidovudine, Nelfinavir, Nevirapine, Lamivudine)
- *Aloe vera* dermal
- *Aloe vera* oral
- Bisphenol A (CFSAN)
- Bitter orange, *Citrus aurantium* (CFSAN)
- Cellular telephone radiation (CDRH)
- Chloral hydrate (CFSAN)
- Di-(2-ethylhexyl)phthalate (CBER, CDRH)
- Ethinyl estradiol (CDER)
- Fumonisin B_1 (CFSAN)
- Furan (CFSAN)
- Genistein (CFSAN)
- Glucosamine/Chondroitin (CFSAN)
- Goldenseal, berberine (CFSAN)
- Ketamine (CDER)
- Malachite green (CVM)
- Melamine with cyanuric acid (CVM)
- Nanoscale silver (FDA)
- Nonylphenol (CDER)
- Oxybenzone (CDER)
- Permanent makeup pigments (CFSAN)
- Retinyl palmitate (CFSAN)
- Riddelliine (CFSAN)
- Triclosan (CDER)
- Urethane/Ethanol (CFSAN)
- Usnic acid, *Usnea* lichen (CFSAN)

The NIEHS/NTP IAG currently supports the NCTR research projects listed below.

- **Acrylamide**—Genotoxicity and carcinogenicity of acrylamide and its metabolite, glycidamide, in rodents (range-finding, subchronic, two-year chronic carcinogenicity studies); developmental neurotoxicity in rats.
- **AIDS therapeutics**—Perinatal carcinogenicity of drug combinations used to prevent mother-to-child transmission of HIV; toxicity studies of combinations in p53 (+/-) haploinsufficient transgenic mice.
- **Berberine**—Pharmacokinetics and photoactivation of this component of goldenseal.
- **Bisphenol A (BPA)**—Determination of the pharmacokinetics in rats and nonhuman primates, physiologically based pharmacokinetics (PBPK) modeling, and subchronic and chronic toxicity in rodents.
- **Bitter Orange (*Citrus aurantium*)**—Developmental and physiological toxicity in rats
- **Cellular telephone radiation**—Histopathological studies on brains from rodents exposed *in vivo* and *in vitro*; effects on rodent blood-brain barrier epithelial cells.
- **Di(2-ethylhexyl)phthalate (DEHP)**—Toxicokinetics in neonatal male rhesus nonhuman primates following intravenous and oral dosing.
- **Furan**—Determination of carcinogenic mechanism and low-dose carcinogenesis in rats.
- **Glucosamine and chondroitin sulfate**—Subchronic toxicity in Fischer 344 rats and diabetic Zucker rats.
- **Melamine and cyanuric acid**—Acute and subchronic toxicity studies and biomarker identification in rodents.
- **Nanoscale silver**—Pharmacokinetics, tissue distribution, and subchronic toxicity in rats.
- **Oxybenzone**—Range-finding studies in rats on developmental toxicity.
- **Retinyl palmitate**—Effect of topically applied skin creams containing retinyl palmitate on the photocarcinogenicity of simulated-solar light (SSL) in SKH-1 mice.
- **Triclosan**—Toxicokinetics and dermal carcinogenicity studies.
- **Usnic acid, *Usnea* lichen**—Toxicity studies in Fischer 344 rats and B6C3F$_1$ mice.

Veterinary Pathology Services Contract

NCTR maintains an on-site pathology contract for veterinary pathology services. The contractor maintains a staff of five board-certified veterinary pathologists and provides NCTR with services from necropsy, clinical pathology and histopathology slide preparation to the rigorous pathology examinations required by the NIEHS/NTP IAG. The work includes translational and applied research that must be performed with rigid adherence to standard operating procedures, NTP specifications, and FDA's Good Laboratory Practice regulations. Extended services include:

- Immunohistochemistry proliferation assays.
    - BrdU IHC
    - Ki-67 (MIB-5) IHC PCNA
    - *In situ* hybridization for histone mRNA apoptosis assays
    - *In situ* hybridization PCR-Solution PCR
    - *In situ* RT-PCR
- Laser-capture microdissection.
- Image analysis using PC-based Optimus and Image Pro Plus image analysis software.
- Imaging system—Virtual Microscopy/Pathology System (ScanScope) for digital storage of microscope slides at diagnostic resolution for local and remote diagnostic collaboration.

Collaborative Research and Development Agreements

NCTR actively pursues and maintains partnerships with nongovernmental organizations, nonprofit organizations, and private companies through Collaborative Research and Development Agreements (CRADAs). The FY 2011 or FY 2012 CRADAs supporting NCTR research projects include those listed below.

Boehringer Ingelheim Pharmaceuticals, Inc.

Pramipexole: Thirty-Week Toxicity Study in Juvenile Rhesus Nonhuman Primates Followed by a Twelve-Week Recovery Period: Use of a Nonhuman Primate Model for Studying the Consequences of Long-Term Dopaminergic Receptor Stimulation on Complex Brain Functions Using the NCTR Operant Test Battery (E0725201)

Cellular Dynamics

Preclinical Studies Investigating the Dose Range and Proof-of-Principle That Leflunomide-Induced Liver Injury Is Enhanced by Cytochrome P450 Inhibition (E0744201)

The Hamner Institutes for Health Sciences

Cancer Mutations as Biomarkers of Cancer Risk: Human Studies with Implications for Personalized Medicine (E0726501)

The Hamner Institutes for Health Sciences

Evaluating the Utility of ACB-PCR in Dose-Response Assessment and Mode-of-Action Evaluation (E0726901)

Toxicology Excellence for Risk Assessment (TERA)

Development of a Method To Use *In vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer Via a Mutagenic or a Non-mutagenic Mode-of-Action (E0722901)

University of Illinois

Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001/21)

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Introduction

In FY 2011, ADRA scientists contributed to research that advanced scientific approaches, personalized medicine, and research tools to support public health. Scientists participated in national and international meetings that promote regulatory and public health issues related to FDA.

ADRA's mission is to:

1) Develop *in vitro* assays using human cells to better understand sex differences, mechanistic- and toxic-action of drugs.
2) Develop biomarkers associated with drug toxicity and carcinogenesis in human tissues.
3) Investigate dietary agents and their mechanisms-of-action in drug efficacy.
4) Investigate the role of epigenetic regulation of metabolic and transporter genes on drug efficacy and chemotherapeutic index.

Scientists have demonstrated their research contributions through the following accomplishments:

- Demonstrated epigenetic changes in the expression of DNA methyltransferases according to ethnicity and sex in patients diagnosed with systemic lupus erythematosus (SLE). These findings suggest that epigenetic changes may play a critical role in the manifestation of the disease observed among ethnic groups, particularly African-American women.
- Demonstrated that -1149 G/T functional single-nucleotide polymorphism (SNP) in the extrapituitary promoter is associated with higher prolactin levels in a cohort of women with lupus.
- Demonstrated that the polymorphic C-to-T change in the promoter region of DNA methyltransferase-3B gene is associated with higher activity in cancer cells, particularly pancreatic-cancer cells. This was the first time that the association was shown in pancreatic-cancer cells, and suggests that DNA methyltransferase-3B may influence risk for pancreatic cancer, since several case-control studies have reported that this polymorphism has been associated with increased risk of cancer.
- Showed that the dietary agent indole-3-carbinol (I3C) in combination with the

anti-pancreatic cancer drug, gemcitabine, enhances cell death through up-regulation of human equilibrative nucleoside transporter 1 (hENT1) in pancreatic-cancer cell lines, but not in normal pancreatic-cell lines. These results suggest that variations in hENT1 expression may at least partially account for inter-individual differences in the clinical effect of gemcitabine and that up-regulation of hENT1 expression may be a promising strategy to improve the effectiveness of gemcitabine.

- Identified 1,640 genes that are differentially expressed according to sex using microarray analyses in human liver. Of these, 26 genes are molecular transporters. Seventeen genes are part of the solute carrier (SLC) family (*SLCO1B1*, *SLC30A1, 43A1, 31A2, 31A1, 35F5, 4A1AP, 5A10, 5A6, 6A16, 9A1, 10A1,16A11, 22A12, 22A2, 22A9, 25A13*) and three genes are part of the ATP-binding (ABC) family (*ABCA5, ABCB6, and ACCN4*). Other transporters that were differentially expressed were *OST* beta, *ATP7B, KCNK5, TRPC4AP, KCNJ8, and ATP2B2*.
- Demonstrated the association of a potential risk biomarker in breast cancer with menopausal status and stage of disease.

FY 2012 Plans

- Study the role of hormones and epigenetics on influencing factors in CYP1A2 expression as potential determinants of sex and individual variation.
- Study the role of sex in expression of histone acetylases, methyltransferases, and demethylases among patients with lupus.
- Study male versus female expression in human liver, kidney, and small intestine using microarray analyses.
- Study sex and ethnic differences in expression of Toll-like receptors in lupus as potential new targets for emerging therapeutics.
- Investigate polymorphisms in the human equilibrative nucleoside transporter 1 in normal and pancreatic-cancer tissues.
- Assess inter-individual variations in expression of human UDP-glucuronosyltransferases and drug transporters in normal, chronic pancreatitis, and pancreatic cancer as mediators of influence on adverse reactions and therapeutics.
- Study the influence of environmental and dietary factors on the effects of tobacco smoke through epigenetic mechanisms.

Women's Health Research

In 2008, NCTR formed a Women's Health Research Group (within the Office of the Director) and a seminar series to promote and coordinate research in women's health within the Center. This group runs an active and innovative research program that focuses on understanding: 1) the molecular basis of drug efficacy and safety, and 2) how genetics, sex, diet, and other environmental factors influence drug efficacy and safety.

This group also coordinates women's health-research projects that are funded by NCTR, FDA's Office of Women's Health (OWH), FDA's Commissioner's Fellowship Program, and extramural grants and partnerships to ensure that the research conducted fills critical knowledge gaps in the safety and efficacy of FDA-regulated products as they relate to sex differences in improving women's health.

In August 2011, NCTR hosted an OWH-sponsored workshop, "Updates and Strategies for FDA Regulatory Impact for 2020: A Vision for the Future." The workshop focused on critical needs for women's health research identified in the Institute of Medicine 2010 report and the Health and Human Services/National Institutes of Health Office of Women's Health 2020 Strategic Plan that also affect FDA regulatory evaluations. Scientists from the NCTR Women's Health Research Group also participated in a workshop sponsored by FDA's OWH and the Society for Women's Health Research on developing successful strategies for engaging women and minorities in clinical trials.

Office of Minority Health

FDA's Office of Minority Health (OMH) Program was started in 2011 as required by the Affordable Care Act. OMH will work to support FDA's mission to protect the public health by assuring the safety, efficacy, and security of human and veterinary drugs, the food supply, biological products, medical devices, cosmetics, radiation-emitting products, and by regulating tobacco. OMH will also work to support the HHS Office of Minority Health's efforts to eliminate racial and ethnic disparities, and to improve minority health and the quality of health care that minorities receive.

FY 2011 Accomplishments and FY 2012 Plans

Minorities are under-represented in clinical research and trials, particularly those in therapeutic areas, which affect minorities disproportionally, such as diabetes, cardiovascular disease, hypertension, stroke, AIDS, and certain cancers (colon, prostate, and cervix). An NCTR scientist from ADRA hosted a round table, which discussed novel approaches for the recruitment, retention, and analysis of research studies on triple-negative breast cancer, a condition with disproportional effects in African-American women.

Moving forward, NCTR will promote and coordinate research studies within NCTR to improve minority health.

NCTR/ORA Nanotechnology Core Facility (NanoCore)

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Introduction

The Nanotechnology Core Facility (NanoCore) at Jefferson Laboratories is a joint effort by NCTR and Office of Regulatory Affairs' (ORA) Arkansas Regional Laboratory (ARL) with a mission to:

Provide the technical expertise and capability to support regulatory research and surveillance needs of NCTR, ORA, FDA, and government agency partners.

Nanotechnology is a multidisciplinary field, drawing from applied and device physics, material science, supramolecular and polymer chemistry, interface and colloidal science, and engineering (chemical, mechanical, biological, and electrical), that involves matter manipulation at the atomic level. Nanotechnology is typically defined as the manipulation of collections of atoms between 1 and 100 nm (0.001 to 0.1 micrometer); however, FDA has not adopted a size-based definition of nanotechnology. The NanoCore support for studies includes: 1) the characterization of the starting materials for toxicology and other studies before and after distribution into solutions and 2) detection of these and other nanomaterials in biological and physical matrices, including studies that investigate the biodistribution, toxicity, or detection of nanotechnology-based materials.

One critical starting point to any study on nanomaterials is characterization of the test article and determination of its behavior in the test environment (*e.g.,* suspension in solution, in food matrix, in water). There is strong agreement that test articles should be characterized for many properties, including average particle size, agglomeration, shape, chemical composition, purity, crystallinity, stability, sterility, endotoxin presence, surface area, chemistry, and surface charge. The NanoCore supports investigators by providing the appropriate equipment, standard operating procedures, and personnel to either conduct these characterizations or train laboratory personnel in conducting the analyses.

FY 2011 Accomplishments

The National Institute of Environmental Health Sciences (NIEHS), National Toxicology Program (NTP) has been instrumental as an agency partner in facilitating the growth of the NanoCore on the Jefferson Laboratories Campus. Several of the studies that have been completed, or are being conducted, are supported through an Interagency Agreement (IAG) between FDA/NCTR and NIEHS/NTP. In addition, 2010 America Reinvestment and Recovery Act funds were provided by NIEHS/NTP to purchase equipment to enable the growth of the NanoCore.

In 2011, the NanoCore expanded its NCTR and ORA personnel to nearly a full complement of staff. Standard Operating Procedures were established for several of the methodologies, and the support on NCTR and ORA projects was initiated.

FY 2012 Plans

The NCTR Strategic Plan outlines specific objectives and outcomes for Goal 1 (*Advance Scientific Approaches and Tools Necessary to Support Public Health*; Objective 1.1, *Integrated Safety Assessment*) with a 2012 Outcome of "*Strengthen the expertise of the NCTR/ORA Nanotechnology Core Facility staff through recruitment of additional experts and establishment of collaborative projects with FDA regulatory centers and the scientific community.*" This goal was met in 2011 with the recruitment of electron microscopy and chemistry staff for particle characterization and elemental detection. This outcome will continue to be developed in 2012 by enhancing collaboration with national partners and developing enhanced methods for nanotechnology support. Studies at NCTR, ORA, CFSAN, and local universities are currently being supported by the NanoCore.

Division of Biochemical Toxicology Summary of Activities

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Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research designed specifically to define the biological mechanisms-of-action underlying the toxicity of products regulated by, or of interest to, FDA. This research centers on quantifying the toxicities and carcinogenic risks associated with specific chemicals and introducing new risk-assessment techniques to enable regulatory agencies to better evaluate the risks associated with exposure to chemicals. The risk-assessment research is firmly rooted in mechanistic studies focused on understanding toxicological endpoints, an approach that allows greater confidence in subsequent risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, analytical chemistry, cellular and molecular biology, nutritional biochemistry, toxicology, phototoxicology, computational risk-assessment methods, and pharmacology. Division investigators work in close collaboration with scientists in FDA Product Centers, the National Institute of Environmental Health Sciences/National Toxicology Program (NIEHS/NTP), and academia to address FDA's regulatory needs.

FY 2011 Accomplishments

A major emphasis within the Division continues to be research on compounds nominated by FDA for evaluation by NIEHS/NTP. This focus reflects NCTR's superb animal facilities supported by a multidisciplinary staff of scientists with strong mechanistic-research experience, which allows subchronic and chronic toxicological assessments to be conducted in a rigorous manner, often adhering to Good Laboratory Practice guidelines. These studies currently serve as the benchmark by which toxicological assessments are made by FDA, other federal agencies, and international regulatory bodies. In addition to providing basic information on toxicological endpoints such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

During FY 2011, an NTP technical report peer-review panel approved the final report for rodent chronic bioassays on acrylamide, a carcinogen found in many baked and fried

foods, which was nominated to NTP by FDA's Center for Food Safety and Applied Nutrition (CFSAN). These data formed the basis for a risk assessment conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) to establish the risk of dietary exposures to acrylamide. In addition, a neonatal mouse bioassay was completed on acrylamide and its oxidized metabolite, glycidamide. The data indicate that the carcinogenicity of acrylamide is dependent upon its metabolism to glycidamide, with the resultant formation of glycidamide-DNA adducts and mutations.

In further investigations of foodborne carcinogens and at the request of CFSAN, experiments were conducted to characterize the risks associated with dietary exposure to furan. These experiments included a large-scale bioassay, encompassing a very wide range of doses, and mechanistic studies that emphasized time- and dose-response relationships in the liver, the target organ for furan's carcinogenicity. The combined bioassay results and mechanistic data will be used to develop physiologically based pharmacokinetic (PBPK) models for furan risk assessments.

During FY 2011, an NTP technical report peer-review panel approved the final report for rodent bioassays on orally administered *Aloe vera,* a widely used dietary supplement. The bioassay results indicated that whole-leaf preparations of *Aloe vera* induced a high incidence of colonic tumors in rats, which share similarities with human sporadic colorectal tumors, at both the morphological and molecular levels.

A major focus within the Division is to elucidate potential toxicities associated with endocrine-disrupting chemicals. Much of this emphasis has been placed on bisphenol A (BPA), an endocrine disruptor to which there is ubiquitous exposure from the environment and food products. This research effort, which is supported by CFSAN, is in response to an increasing concern over BPA due to a large and growing body of literature reporting nonmontonic dose-response effects of BPA at doses approaching human-exposure levels. During FY 2011, Division investigators completed a subchronic study of BPA in rats that used a broad range of doses administered orally to pregnant dams and directly to the pups after birth. Endpoints related to reproductive development, control of energy utilization, and general toxicity were evaluated. In addition to conducting a subchronic study, manuscripts were published describing the pharmacokinetics of BPA administered orally to rodents, nonhuman primates, and humans. PBPK models for BPA have been developed to reduce uncertainty in extrapolating any effects seen in experimental animals to those possible in humans.

During FY 2011, studies were conducted to investigate the toxicities of topically applied triclosan, a broad-spectrum antimicrobial agent present in a wide variety of antibacterial soaps, deodorants, toothpastes, cosmetics, fabrics, plastics, and other products, which was nominated by the Center for Drug Evaluation and Research (CDER). These experiments included pharmacokinetic analyses to measure the dermal absorption of triclosan and to characterize the metabolites formed, as well as a subchronic study.

Antiretroviral drugs are used to prevent the mother-to-child transmission of human immunodeficiency virus type-1 (HIV-1), the virus responsible for AIDS. While these drugs are effective in preventing the viral transmission of HIV-1, the long-term consequences of perinatal exposure to these drugs are presently unknown. During FY 2011, an NTP technical report peer-review panel approved the final report on rodent bioassays in which antiretroviral drugs were administered transplacentally to mice. In addition, a manuscript was published characterizing the oxidation products of 2-hydroxynevirapine—a metabolite of the antiretroviral drug, nevirapine.

The intentional adulteration of pet food with melamine and derivatives, including cyanuric acid, has been implicated in the kidney failure and death of a large number of cats and dogs in the U.S. While individually these compounds present low-toxicity, co-exposure can lead to the formation of melamine-cyanurate crystals in the nephrons of the kidney and eventual kidney failure. During FY 2011, investigators in the Division, in collaboration with colleagues at FDA's CFSAN and Center for Veterinary Medicine (CVM), conducted experiments in rats to determine the dose-response for nephrotoxicity upon co-administration of melamine and cyanuric acid. The data indicate that tolerable daily intakes, based upon studies conducted with melamine alone, may underestimate the risk from co-exposures to melamine and cyanuric acid. Pharmacokinetic analyses were also performed, which enabled a better understanding of the risks associated with particular scenarios of exposure, and in collaboration with scientists at FDA's Center for Devices and Radiological Health (CDRH), genomic and noninvasive biomarkers associated with kidney toxicity were investigated to expand the number of endpoints that can be used to assess melamine and cyanuric acid-induced kidney toxicity.

In the event of a bioterrorism attack on a food-production facility, chemical decontamination methods will be needed that have been tested and proven to be effective. Investigators within the Division, along with collaborators at CFSAN, compared the efficacies of sodium hypochlorite (household bleach), peroxyacetic acid, and other chemical agents used by the food industry to inactivate the bioterrorism agents, ricin and abrin, in dried food residues adsorbed to food-contact surfaces. The results, which formed the basis of a final report to the National Center for Food Protection and Defense during FY 2011, showed rapid, complete, and irreversible inactivation of ricin and abrin on food-contact surfaces by sodium hypochlorite and slower or incomplete toxin inactivation by the other chemical agents. Infants and very young children are considered to represent a vulnerable target population for foodborne bioterrorism agents because they are prone to consume a single type of food as a complete meal (*e.g.*, baby foods or infant formulas). In subsequent studies, investigators in the Division compared the thermal stability of the toxin ricin in milk, milk-based infant formula, soy-based infant formula, four types of fruit juices, a yogurt-fruit blend marketed for toddlers, and plain yogurt. The stability of ricin was similar in most of the dairy or fruit products tested, but blended yogurt-fruit drink enhanced the thermal stability of ricin, an effect that was partially duplicated by plain yogurt. In further experiments,

investigators in the Division addressed a strategic goal of FDA by developing an improved method for detecting the biochemical activity of ricin, abrin, and the shiga-like toxins in dairy products. Division investigators also collaborated with NCTR food microbiologists to develop sensitive enzyme-linked immunosorbent assay (ELISA) methods to detect and quantify shiga-like toxins produced by *E. Coli* O157:H7 and related pathogenic bacterial strains found in foods.

Silver nanoparticles are highly effective antibacterial agents, and this property of silver nanoparticles is being exploited in an expanding number of commercial and consumer products. Human exposure to silver nanoparticles continues to increase with every new application, which has led to public concerns regarding the safety of silver nanoparticles. Consequently, CFSAN nominated silver nanoparticles for an in-depth toxicological evaluation. During FY 2011, Division investigators examined the effect of the size of silver nanoparticles on the bioavailability, tissue distribution, metabolism, and clearance in rats. The results indicated that, after oral administration, only limited absorption occurs, with the extent of absorption increasing as the size of the particle decreased. In a subsequent study, a subchronic bioassay was initiated to determine the toxicity associated with repeated oral administration of silver nanoparticles.

A strong emphasis within the Division has been to determine whether epigenetic changes (*e.g.*, DNA methylation) induced by carcinogens, and found in tumors, play a causative role in carcinogenesis or are merely a consequence of the transformed state. During FY 2011, Division investigators—in collaboration with academia—assessed the potential role of epigenetic changes as early predictive markers for toxicity and carcinogenicity safety assessments. They demonstrated that short-term inhalation of the genotoxic carcinogen 1,3-butadiene, in addition to inducing the formation of DNA adducts, also caused prominent epigenetic alterations in the liver of mice. These results highlight the significance of epigenetic events in the mechanism of 1,3-butadiene toxicity and carcinogenicity. Also, investigators in the Division demonstrated that differences in interindividual susceptibility to 1,3-butadiene and 1,1,2-trichloroethylene may be due to variations in epigenetic responses. These findings suggest that the assessment of carcinogen-induced epigenetic alterations, in addition to genetic changes, may substantially improve the safety evaluation of FDA-regulated products and facilitate novel approaches to identify subpopulations susceptible to exposures.

Herbal products represent the fastest growing segment of the vitamin, mineral supplements, and herbal-products industry because of the belief that they are beneficial to health. Although these "natural" products are perceived as safe, evidence from clinical and experimental studies suggests that the use of herbal dietary supplements is not without risk. One such dietary supplement is Kava (*Piper methysticum*), a plant used for its presumed sedating and relaxing properties. Division investigators examined kava-associated phototoxicity, kava-drug interactions, the individual constituents responsible for the toxicity of kava, and the underlying molecular mechanisms of kava toxicities.

FY 2012 Plans

In FY 2012 Division of Biochemical Toxicology investigators will:

- Prepare an NTP report on the perinatal carcinogenicity of antiretroviral drugs.
- Prepare an NTP report on two-year chronic rodent bioassays of glycidamide, a toxic metabolite of acrylamide.
- Complete the in-life phase of a chronic bioassay of the food contaminant furan.
- Complete a subchronic study to determine the toxicities associated with exposure to silver nanoparticles.
- Complete a subchronic study to investigate the toxicities of topically applied triclosan.
- Initiate a two-year chronic bioassay to characterize the toxicities of BPA in rodent models, with special emphasis on perinatal exposures.
- Develop PBPK pregnancy models for rodents, nonhuman primates, and humans to predict placental transfer of BPA to the fetus.
- Assess the effects of intravenous exposure to di (2-ethylhexyl) phthalate (DEHP), a commonly used plasticizer in medical devices, in the lungs and hearts of neonatal rats.
- Complete a subchronic study to evaluate the toxicities of melamine in combination with cyanuric acid in adult rats.
- Conduct a subchronic study to assess the toxicities of melamine, cyanuric acid, and their combination in newborn rats. The goal of this experiment is to determine if the developing kidney in newborn animals is more susceptible to the nephrotoxic effects of these dietary contaminants.
- Compare the relative sensitivities of multiplex polymerase chain reaction (PCR), ELISA, toxin-dependent enzyme activity assays, RAPID-B™ technology, and conventional bacterial plate count methods for the rapid detection of pathogenic enterohemorrhagic *E. Coli* strains in foods during the pre-enrichment culture process.
- Test the hypothesis that standard pasteurization conditions approved for milk are inadequate for complete inactivation of the bioterrorism agent, ricin, and test the efficiency of sodium hypochlorite solutions to inactivate ricin-contaminated milk pasteurization equipment.
- Initiate subchronic studies with *Aloe vera* whole-leaf preparations that have been treated with charcoal and with *Aloe vera* gel. The goal of these experiments will be to determine if *Aloe vera* subfractions give the same toxic responses as *Aloe vera* whole-leaf preparations.

- Initiate a one-year photo-cocarcinogenicity study on retinyl palmitate, a component of skin care and cosmetic products.
- Investigate the potential of pyrrolizidine alkaloid-DNA adducts to be used as biomarkers of pyrrolizidine alkaloid exposure and tumorigenicity.
- Investigate the role of epigenetic and microRNA alterations in blood plasma as potential biomarkers for noninvasive evaluation of exposure to genotoxic and nongenotoxic compounds of interest to FDA.
- Initiate studies to evaluate the correction of epigenetic abnormalities as a novel approach for personalized cancer prevention.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Biochemical Toxicology contributes to NCTR Strategic Goal 1 (Advance Scientific Approaches and Tools Necessary To Support Public Health), which supports FDA Strategic FDA Strategic Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*); Goal 3.1 (*Advance Food Safety and Nutrition*), and Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*) under the following NCTR objectives:

Objective 1.1: Integrated safety assessments

A major emphasis of the Division's research is to ensure the safety of food products. This is accomplished in close coordination with CFSAN and other FDA Product Centers, which identify research needs and data gaps that guide the design of the Division's studies. For example, Division investigators are conducting bioassays and mechanistic studies to assess the risk of dietary exposures to acrylamide, a known rodent carcinogen and neurotoxicant that has been identified in baked and fried starchy foods—notably french fries, potato chips, bread, coffee, and many other consumer food products. A similar research strategy is being applied to furan, another contaminant in food. Evaluations are also being conducted on BPA, a chemical derived primarily from food-contact uses to which there is ubiquitous environmental exposure, and on *Aloe vera*, a natural product incorporated into dietary supplements. As part of the Division's efforts to ensure the safety of foods, assays are being developed and applied to detect the biological activities of potential bioterrorism agents, such as ricin and abrin, in various food products. Division investigators are also conducting studies to assess the toxicities associated with exposure to melamine, cyanuric acid, and pyrrolizidine alkaloids, contaminants that have been found in certain food products. In addition, Division researchers are assessing the effects of intravenous DEHP in a neonatal rodent model. This experiment addresses the human exposure to DEHP of highest concern—exposure occurring in neonatal intensive-care units due to leaching of DEHP from medical devices.

Objective 1.2: Advance emerging science through the development and application of new tools and approaches

Computational tools are being developed to integrate toxicological, mechanistic, and pharmacokinetic data for safety assessments. PBPK models of BPA in nonhuman primates and humans have been developed to reduce the uncertainty in predicting human-health risks from exposure to BPA. Biologically based dose response (BBDR) models for the thyroid axis are being developed for the human fetus/child and the rodent fetus/pup for thyroid active chemicals (e.g., perchlorate and thiocyanate) found in food. The hypothalamus-thyroid axis BBDR models will help estimate human biologically equivalent doses associated with thyroid disturbances observed in rodents administered perchlorate or thiocyanate.

Division of Genetic & Molecular Toxicology Summary of Activities

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Introduction

The Division of Genetic and Molecular Toxicology conducts basic and applied research to address specific high-priority issues related to the induction of genetic damage. Division research is directed toward developing and validating new methods or improving existing methods to identify potentially hazardous food additives, human and animal drugs, biological therapies, and medical devices. In collaboration with other FDA scientists, the Division utilizes the methodologies it develops to better understand the potential toxicity of specific high-priority drugs, dietary supplements, and other agents. As experts in the field of genetic toxicology, scientists in the Division are actively involved in national and international efforts to harmonize the conduct of genetic-toxicology tests and to improve their interpretation and use for regulatory decision making. Division scientists are actively participating in the Organization for Economic Cooperation and Development (OECD) expert workgroup that is revising the current Genetic Toxicology Guidelines and developing guidelines for assays that currently do not have guidelines. Division scientists frequently provide expert advice to FDA Product Centers, other government agencies, academia, and industry. They also are active participants in the FDA Genetic Toxicology Network, and other interagency workgroups.

The Division's research is divided into four themes:

1) Research Involving Current Regulatory Genetic-Toxicology Assays

2) Chemical-Specific Research

3) Development of New Assays and Approaches

4) Research to Improve Risk Assessment

FY 2011 Accomplishments

In FY 2011, Division of Genetic and Molecular Toxicology scientists actively participated in providing genetic-toxicology advice to FDA Product Centers. These consultations included general advice concerning the conduct and interpretation of data from specific assays, as well as evaluation of data from FDA submissions. Division scientists participated in various Genetic Toxicology Working Groups of several organizations, including the International Workshop for Genotoxicity Testing (IWGT), the OECD, and

International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI). Division scientists were, and will continue to be, involved in discussions concerning the appropriate strategies for evaluating chemicals for product safety.

Specific FY 2011 research accomplishments involving the current regulatory assays include:

- In direct response to an FDA need, a project was continued to evaluate various measures of cytotoxicity and other assay parameters for the *in vitro* micronucleus assay. The project also compared two different cell lines and also gained experience with the flow-cytometric method to evaluate micronucleus formation.

- Continued a study to gain expertise in the comet assay and conduct research to evaluate the important parameters of this technique. The ultimate goal of this project is to provide information and expertise that can be used to help develop guidance documents for the conduct of this assay.

- Published the conclusions from the IWGT expert workgroup of the Mouse Lymphoma Assay.

- Published the conclusions from the IWGT expert workgroup for *in vitro* cytogenetic assays.

- Expanded a study to evaluate whether the current genetic toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.

- Established protocols for evaluating the standard genetic toxicology test for their applicability to assessing the hazards of nanomaterials.

- Completed experiments, wrote, and submitted a manuscript describing initial studies evaluating the mutagenicity of nanosilver.

- Competed for and received funding from the Office of the Commissioner's Collaborative Opportunities for Research Excellence in Science (CORES) research program for nanomaterials.

- Served as a member of the FDA Taskforce for nanomaterials.

- Participated in NCTR Science Advisory Board site visit for the NCTR NanoCore and nanomaterial research.

The Division was actively involved in research addressing specific chemicals and generating data that can be used by the FDA Product Centers.

- Completed a study to evaluate the cancer mode-of-action for furan.
- At the request of an FDA Center for Drug Evaluation and Research reviewer, initiated research to investigate the potential mutagenicity of an impurity often seen in pharmaceuticals.
- At the request of an FDA Center for Food Safety and Nutrition (CFSAN) reviewer, initiated and completed research to investigate whether the samples of *Aloe vera* used as a part of the National Toxicology Program (NTP) can induce mutations.
- In collaboration with CFSAN scientists, conducted research and published a manuscript describing the toxicity of methyleugenol.
- In collaboration with FDA's Center for Tobacco (CTP), created a project to develop 3-D cell-culture models to assess the toxicity and inflammatory capabilities of various tobacco products.
- In collaboration with CTP, developed and received funding for a project evaluating genetic-toxicology assays for their ability to distinguish the relative hazards of various tobacco products.

Substantial progress was made in FY 2011 to develop new methods and bring new methodologies to NCTR.

- Completed a project to breed a new double-transgenic mouse that combines the hairless genotype with the gpt delta transgenic-mouse model for use at NCTR to evaluate compounds for their potential to cause skin cancers.
- Completed a project to establish chromosome-painting technology at NCTR.
- Participated in an international trial of a new approach for directly analyzing mutations. This assay uses fluorescent probes to detect mutation in the endogenous X-linked PIG-A gene. The detection of mutations in this gene does not require cell culture (as do many other *in vivo* mutation-detection methods) and lends itself to both *in situ* and high-throughput analyses in humans and animal models. These properties make PIG-A an attractive reporter-gene for *in vivo* mutation studies.
- Completed a series of publications on various aspects of the development of the PIG-A gene mutation assay. These publications were part of a complete issue of

Environmental and Molecular Mutagenesis devoted to the PIG-A assay. A Division scientist was a co-editor of this special issue.

- Completed research and published a manuscript describing the use of cancer biomarkers to evaluate aristolochic acid-induced carcinogenicity.

- In collaboration with NCTR's Division of Systems Biology (DSB), published a paper describing the use of urinary microRNAs as noninvasive biomarkers for acetaminophen-induced liver injury.

- In collaboration with DSB, published a paper using microRNA expression profiles to distinguish the carcinogenic effects of riddelliine in rat liver.

- In collaboration with DSB, published a paper comparing next-generation sequencing and microarray technologies in a toxicological study of the effects of aristolochic acid on rat kidney.

- Completed research and published a manuscript describing a functional comparison of microarray data across multiple platforms using the method of percentage of overlapping functions.

The Division conducted research to improve risk assessment in FY 2011.

- Conducted research that is a part of a large multi-organization project to use *in vivo* mutation analysis to inform cancer mode-of-action for ethylene oxide inhalation. This project is funded by a Cooperative Research and Development Agreement (CRADA) with Toxicology Excellence for Risk Assessment (TERA).

- Completed a portion of a project to investigate whether mutagens can have thresholds. This study revealed that the shape of the dose-response curve can vary depending on the mutational endpoint used and also on the tissue evaluated. In addition, the study provided evidence that there may be a difference between the shape of the dose-response curve of neonates and adult animals.

- Continued research using an allele-specific competitive blocker-polymerase chain reaction (ACB-PCR) technology and progress indicates that this approach provides the opportunity to detect the rare mutations involved in the etiology of cancer prior to the development of the actual visible tumor. This appears to be a promising biomarker that may provide a strategy that might ultimately lead to replacing the traditional two-year cancer bioassay and hasten the development, safety assessment, and approval of new drugs.

- Conducted research to investigate whether the ACB-PCR methodology can be applied to detecting subpopulations of specific mutations in tumors, and

thereby, be a useful tool in distinguishing individuals who will benefit from particular cancer therapies from those that will not. This project has the potential to advance personalized medicine.

- Completed research as part of a CRADA with the Hamner Institute and published a manuscript using cancer biomarkers to demonstrate that naphthalene does not increase p53 mutations in nasal respiratory and olfactory epithelia of rats.

FY 2012 Plans

The Division will continue research in all four theme areas. Specific plans include:

- Gain expertise in the comet assay and conduct research to evaluate the important parameter of this technique. The ultimate goal of this project is to provide information and expertise that can be used to assist with the development of guidance documents for the conduct of this assay.
- Initiate project using the new *in vitro* 3-D skin assay.
- Evaluate whether the current genetic-toxicology assays are appropriate for evaluating the potential toxicity of nanomaterials.
- Assess whether there is a difference in the mutagenic potential of nanosilver particles and bulk silver.
- Evaluate the relative mutagenic potential for tobacco products in collaboration with FDA's Center for Tobacco Products (CTP).
- Initiate a new study in collaboration with CTP to use new 3-D tissue models to assess the relative cytotoxicity/inflammatory response for different tobacco products.
- Evaluate the cancer mode-of-action for ethylene oxide.
- Validate further, a new approach for directly analyzing mutations. This assay uses fluorescent probes to detect mutation in the endogenous X-linked PIG-A gene.
- Evaluate whether microRNA expression analysis can be used to detect carcinogens from noncarcinogens.
- Establish and evaluate the gpt delta transgenic-mouse model for use at NCTR.
- Research using an ACB-PCR (allele-specific competitive blocker-polymerase chain reaction)-technology. Progress indicates that this approach provides the opportunity to detect the rare mutations involved in the etiology of cancer prior to the development of the actual visible tumor.

- Direct a new research effort toward understanding the background frequency of these cancer mutations in “normal” individuals. This will include the potential impact of rodent strain and age of the rodents. In addition, efforts will be made to make the technology more rapid and easy to conduct.

- Investigate whether mutagens can have thresholds, thus evaluating whether there may be doses below which exposure to mutagens/carcinogens will have no adverse genetic effects.

Contributions to FDA’s Strategic Priorities/Goals

The research conducted by the Division of Genetic and Molecular Toxicology contributes to NCTR Strategic Goal 1 (*Advance Scientific Approaches and Tools Necessary to Support Public Health*) and to FDA Strategic Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*); Goal 3.1 (*Advance Food Safety and Nutrition*), and Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*).

The Division provides expert advice and innovative research to the FDA Product Centers, thus contributing to FDA’s mission of advancing public health. Several research projects involve the development of new and innovative technologies and approaches that support FDA’s Product Centers and, in particular, the FDA Critical Path Initiative.

Genetic toxicology is concerned with the ability of chemicals to alter genetic material. FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product-approval process. Because genetic damage is believed to be important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode-of-action. Research within the Division focuses on the development and validation of new methods to assess genetic risk. Bacterial and tissue-culture approaches are commonly used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity. While the Division utilizes *in vitro* approaches, it specializes in the development and validation of *in vivo* mammalian systems and the incorporation of these methods into risk-assessment strategies. An increased understanding of mutational mechanisms, combined with test systems that have an increased ability to detect genetic damage, will provide FDA with better information for decision making. As new assays are validated, Division scientists will continue to work with international scientists to assure the harmonization of protocols and the development of guidelines to assess genetic hazards.

Genomic technologies are beginning to provide new tools for making better public-health decisions. International research efforts are providing the scientific and medical community with an increased understanding of the genetic material and how it

functions in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes to the genetic material of both rodents and humans. The Division is using new technologies, in combination with more traditional approaches, to address various research questions. While current technologies in the field of genetic toxicology generally evaluate single endpoints, the new genomic technologies are providing the opportunity to detect alterations in a number of endpoints. In the future, these new approaches to evaluate toxicity will allow for the integration of information across the various types of adverse-health outcomes. For instance, when these technologies are fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects, and to modify the immune function.

Division of Microbiology Summary of Activities

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Introduction

The Division of Microbiology goals are to 1) perform fundamental and applied research to address critical issues in support of the FDA mission and 2) provide technical-service support in responding to microbial surveillance and diagnostic needs for research using experimental animals at NCTR. The Division of Microbiology research projects are based on expertise of division staff and consultation with scientists from academia, industry, and FDA Product Centers. The research program is divided into four focal areas:

1) Food Safety, Food Biosecurity, and Methods Development
2) Antimicrobial Resistance
3) Microbes and Host Interactions
4) Environmental Biotechnology

During FY 2011, the Division of Microbiology scientists engaged in research addressing a variety of FDA issues with special emphasis on:

- Developing rapid technologies to detect, identify, and characterize foodborne pathogens.
- Determining antimicrobial resistance and virulence mechanisms of microbial pathogens that may enter the food supply.
- Using state-of-the-art molecular biological approaches to monitor interactions between human-intestinal microbiota, antimicrobial agents, food contaminants, food additives, and food supplements.
- Conducting studies impacting women's health and well-being.
- Improving environmental risk assessments of priority pollutants, including polycyclic-aromatic hydrocarbons and drugs by integrating systems biology approaches.

FY 2011 Accomplishments

Food Safety, Food Biosecurity, and Methods Development

- Research under an Interagency Agreement (IAG) with the U.S. Department of Agriculture and Department of Homeland Security on the survival of *Bacillus Anthracis* in processed liquid-egg media was completed. The data may help evaluate the benefits and risks of lysozyme addition to foods and improve

biosecurity from deliberate contamination of foods with *B. Anthracis*.

- A multiplex real-time polymerase chain reaction (PCR) method, which was developed for *Salmonella* detection in foods, indicates resistance to several antibiotics. The method is being validated in collaboration with ORA laboratories.

- Coronavirus genomic RNA levels were shown to be stable up to 30 days on lettuce and infectious virus was recovered up to two weeks. Because coronaviruses were stable during the shelf-life of lettuce, they may be transmitted to humans.

- An *in vitro* macrophage and epithelial-cell virulence assay was developed to characterize pathogenicity of bacterial strains isolated from contaminated food.

- Oral challenge experiments using chickens determined that a poorly colonizing *Campylobacter Jejuni* isolate was reduced relative to a robust colonizer in the chicken cecum. Proteins were extracted for whole-proteome analysis.

- Plasmid-associated genes for antimicrobial resistance and virulence in *Salmonella enterica* from poultry and human infections were detected and the plasmids were sequenced to further characterize resistance and virulence. Antimicrobial-resistance profiles and integrons were identified in multidrug-resistant *Salmonella* isolates that persist in organically raised chickens.

- *Salmonella enterica* strains resistant to ciprofloxacin were isolated from imported foods and characterized for virulence genes, plasmids, and mutations in the quinolone-resistance determining region (QRDR).

- In studies of the genetic mechanisms of fluoroquinolone and extended-spectrum β-lactamase resistance in *E. Coli* isolates from companion animals, researchers identified novel mutations that play a role in resistance.

- A transcriptomic microarray analysis was developed for the detection of 131 antimicrobial-resistance genes from multidrug-resistant organisms from poultry, other animals, and clinical sources.

- Microarray and transcriptome analysis of gene expression in wild types and fluoroquinolone-resistant mutants of *Clostridium perfringens* revealed both down-regulation and up-regulation of toxin genes in two resistant mutants.

- Fluoroquinolone-resistant *Aeromonas* mutants from imported shrimp contained double or single mutations in the QRDR of DNA gyrase. These results indicate that imported shrimp may be a reservoir of virulent drug-resistant aeromonads.

Microbes and Host Interactions

- Division staff participated in the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medical Products (VICH) Expert Working Group organized by CVM to evaluate the VICH GL36 guideline on the human safety of veterinary antimicrobial drugs, including assessments of the impact of drug residues in meat on human-intestinal microbiota.

- Research on the bioavailability of antimicrobial residues in foods of animal origin and the potential to induce resistance in the colonic bacteria was completed.

- Molecular study of human coronaviruses suggested that three strains circulating during the last influenza season could have been associated with the acute respiratory symptoms observed in 13% of patients negative for influenza virus.

- Sudan and Para Red dyes selectively inhibit some human-intestinal bacteria, suggesting that dyes and their metabolites could affect the human microbiota.

- Division scientists completed a Cooperative Research and Development Agreement (CRADA) with Pfizer to study degradation of the veterinary antimicrobial ceftiofur by bovine intestinal microflora. Bacteria from cattle not treated with cephalosporins produced β-lactamases and inactivated ceftiofur.

- Cytolethal distending toxin b (*cdtb*) gene, specific for *Salmonella Typhi*, is responsible for persistent infection. Division scientists found that the *cdtb* gene is also present in some non-typhoidal *Salmonella* serovars. A macrophage virulence assay was developed to characterize interaction of *cdtb* gene with host cells; *cdtb* initiates the DNA damage response in host cells as soon as it invades.

Office of Women's Health Projects

- Division scientists found that *Staphylococcus aureus* metabolized Orange II and Sudan III dyes. Sudan III inhibited growth and viability of the skin bacterium. This study examined the potential effects of azo dyes permitted for use in cosmetics.

- Lysostaphin, which degrades the cell wall of *Staphylococcus aureus,* was cloned into *Lactobacillus plantarum* and shown to be bactericidal for toxic shock syndrome toxin-1 (TSST-1) producing strains of *S. aureus* in a medium simulating vaginal secretion. Lysostaphin-producing *Lactobacillus* could potentially be used as a probiotic to inhibit TSST-1 producing *S. aureus*.

- Estrogen and phytoestrogens from soy products, suppressed expression of pro-inflammatory cytokines and signal-transduction genes by vaginal-epithelial cells in response to *Candida Albicans* yeast infection. Estrogen induced the cells to produce hydrogen peroxide to inhibit *C. Albicans*, but enhanced peroxide resistance in the yeast and potentially exacerbated vaginal yeast infections.

Environmental Biotechnology

- Division scientists studied the bacterial metabolism of genotoxic high-molecular weight polycyclic-aromatic hydrocarbons in proposed metabolic pathway networks, based on genomic, proteomic, metabolic, and bioinformatic data.
- Division scientists showed that amino acids enhanced the conversion of the antibiotic norfloxacin by a *Microbacterium* sp. isolated from wastewater to an inactivated product, *N*-acetylnorfloxacin.

Surveillance and Diagnostic Program

The primary mission of the Surveillance and Diagnostic program is to provide assurance that NCTR research data is not compromised by infected or unhealthy experimental animals. During FY 2011, program personnel worked to prevent the introduction of microbial pathogens into NCTR animal colonies. Routine monitoring of the animals, environment, food, and water from the breeder colonies was conducted. Quarantine and sentinel mice and rats were screened for potentially pathogenic microorganisms.

FY 2012 Plans

Food Safety, Food Biosecurity, and Methods Development

- Initiate quantitative proteomic, transcriptomic, and phenotypic microarray analyses of *Campylobacter Jejuni to* identify colonization factors for poultry.
- Develop an infectivity assay to detect human noroviruses in contaminated food.
- Study the expression of bacterial Shiga toxins, using *in vitro* enzyme assays, and compare the cytotoxicities of non-O157:H7 Shiga toxin producing *E. Coli* (STEC) strains to cultured murine macrophages and Vero cells.
- Continue *in vitro* assessments of the impact of plasmid-associated genes on the ability of *Salmonella* to invade intestinal epithelial cells and macrophages.
- Develop detection methods for *Bacillus cereus* in dietary supplements using chromogenic agar media and PCR identification of enterotoxigenic *B. cereus.*
- Elucidate the mechanism of cell death by non-typhoidal *Salmonella* containing Cytolethal Distending Toxin B and identify biomarkers of intestinal inflammation.
- Compare the genomes of *Clostridium perfringens* food isolates with those from soil, animal, and clinical sources by pulsed field gel electrophoresis.

Antimicrobial Resistance

- Elucidate the mechanisms of potential transfer of antimicrobial-resistance genes from resistant *E. Coli* strains, isolated from dogs and cats, to susceptible isolates and study the mechanisms of carbapenem resistance in these isolates.

- Quantify the effect of exposure of *Salmonella* to antimicrobials used in poultry on the efficiency of transfer of plasmids containing multidrug-resistance and virulence determinants. Characterize genes involved in resistance and virulence by plasmid sequencing and macrophage/epithelial-cell virulence assays.

- Study mechanisms for resistance to tigecycline and tetracycline and the role of efflux pumps in antibiotic-resistant *Salmonella enterica* isolated from imported food.

- Study the antimicrobial properties of metal oxide (CaO, MgO, ZnO) nanoparticles and their toxicity to human keratinocytes and gastrointestinal cell lines.

- Evaluate the effect of fluoroquinolones-resistance development in *Clostridium perfringens* on the alteration of transcription in resistant mutants.

- Continue to characterize fluoroquinolone-resistant *E. Coli* and *Vibrio* isolates from imported shrimp for virulence and fluoroquinolone-resistance genes.

Microbes and Host Interactions

- Express coronavirus proteins in baculoviruses and screen human serum for antibodies against α, β and γ-coronaviruses to estimate seroprevalence.

- Continue studies on the bioavailability of antimicrobials and the impact of antimicrobial residues on the human-intestinal microbiota.

- Determine the effect of smokeless tobacco on oral and intestinal bacterial ecology and compare the toxicity before and after metabolism by oral bacteria.

- Evaluate the role of obesity in modulating intestinal bacterial populations capable of metabolizing the soy isoflavone daidzein to equol, which may influence breast-cancer development.

- Determine the inhibitory capabilities of a *Lactobacillus plantarum* strain expressing lysostaphin for staphylococci in a vaginal tract model.

- Identify factors produced by *Lactobacillus* that inhibit staphylococcal extracellular proteins.

- Continue evaluation of the mechanism by which lactobacilli prevent suppression of immune responses against *Salmonella*.

- Culture human-epithelial cells to determine gene-expression profiles after infection by *Bacillus Anthracis*. The effect of infection on expression of human genes will also be studied to identify time-specific gene-expression differences.

- Determine the effect of nanomaterials on the permeability of intestinal epithelial cells and the effect of AgNP on intestinal microbiota of Sprague-Dawley rats.

- Elucidate probiotic and phytoestrogen suppression of pro-inflammatory cytokine responses in vaginal-epithelial cells challenged with *Candida Albicans*.

Environmental Biotechnology

- Examine the impact of BP Deepwater Horizon crude oil on human microbiota, emphasizing polycyclic aromatic hydrocarbons (PAHs) and microbial physiology, as well as the toxicity of crude oil and PAH mixtures to fish, oysters, and shrimp.
- Identify environmental microorganisms in wastewater that inactivate residues of fluoroquinolone antibiotics and their transformation products.

- Isolate and identify the enzyme from *Microbacterium* sp. for *N*-acetylation and inactivation of fluoroquinolones; clone and sequence the corresponding gene.

Microbiological Surveillance and Diagnostic Support of Research

- Ensure that the research-animal population remains healthy and disease-free.

- Develop and use real-time and conventional PCR assays and other new assays.

- Update the MIDI microbial-identification system for the surveillance program.

- Train lab personnel on new technologies and methods in microbial diagnostics.

- Study the feasibility of participation in a national surveillance program's surge capacity.

Contributions to FDA's Strategic Priorities/ Goals

Research in the Division of Microbiology contributes to FDA Strategic Goal 3.1 (*Advance Food Safety and Nutrition*), Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*), and Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*).

- Molecular typing methodologies, involving next-generation genotyping combined with high-efficiency target-capturing methods, can be useful in high-throughput characterization of foodborne pathogens.

- The Genomics Knowledgebase for Enteric Pathogens will link data analysis with biological relevance of pathogens for FDA staff. It can serve as a valuable tool to share information about bacterial isolates and outbreak threats.

- Rapid, sensitive molecular-biology tools are essential for tracking drug-resistant pathogens in imported seafood and aiding FDA in regulating imports.

- Better methods to confirm the presence of infectious noroviruses in foods will improve food safety and assist FDA in resolving legal impasses.
- Investigating the effects of fluoroquinolones on pathogenic bacteria, particularly mutations associated with antibiotic resistance, is valuable for understanding the emergence of more virulent strains of bacteria after fluoroquinolone treatment.
- Metabolism of veterinary antimicrobials by intestinal microbiota and wastewater bacteria may affect development of resistance. Since FDA sets drug-residue limits for animal products, this will assist strategies for antibiotic use.
- Metabolism of isoflavones in soybean-containing diets by the intestinal microbiota may reduce breast cancer. Knowing how obesity affects metabolism will assist FDA in developing guidelines for the consumption of soy products.
- Interaction of nanomaterials with gastrointestinal surfaces will lead to identification, quantitation, and molecular characterization of gut-mucosal exposure to nanomaterials in foods and other FDA-regulated products.
- Rapid detection of Shiga toxins in foods will be critical in preventing outbreaks of *E. Coli* O157:H7 and non-O157:H7 Shiga-toxin producing *E. Coli*.
- Characterization of multidrug-resistant *Salmonella* isolates from imported foods will allow better understanding of the transfer of virulence and drug-resistance plasmids to assess risk of antibiotics in food production. Studies on *Salmonella Javiana* and non-0157:H7 Shiga-toxin producing *E. Coli* will provide data for an FDA risk-assessment model for foodborne pathogens.
- Coronavirus research will increase preparedness for outbreaks and for reviews of vaccines and diagnostic tests for organ transplants and blood transfusions.
- Research on influenza viruses will accelerate the availability of effective and rapid influenza diagnosis.
- Research on susceptibility of gut microbiota to food additives and drugs will have a regulatory impact, because warnings on packages will aid in reducing diseases associated with food and provide nutritional information for a healthy diet.
- Epidemiological, antimicrobial resistance and pathogenicity data from food-safety studies should be useful in assessment of the threat of accidental or deliberate foodborne outbreaks and help prevent the spread of resistance.
- FDA will gain a clearer understanding of how drug residues, probiotics, dietary supplements and xenobiotics affect the intestinal microflora and human health.
- Genetic analysis of bacteria from various sources will assist in determining the route of food contamination. Understanding factors contributing to antimicrobial resistance and virulence in foodborne bacteria will help to develop intervention strategies to improve the safety of foods.
- Environmental biotechnology research will help document environmental fate and

toxicity before FDA approval of medical products. According to CDER Guidance for Industry "Environmental Assessment of Human Drug and Biologics Applications" and CVM Guidance for Industry #89 (2001 Docket No. 99D-2975) and #166 (2006 Docket No. 2004D-0156), degradation is important in environmental assessment of drugs.

- Probiotics are regulated by the Dietary Supplement Health and Education Act of 1994, which focuses on adulteration or health risks. If research on the effects of supplements on pathogens suggests that lowered disease resistance is likely, the supplement should be evaluated for safety. Knowledge of vaginal probiotics may be useful for FDA to assess the efficacy of other probiotics.
- Responses of cutaneous and intestinal epithelial cells to *Bacillus Anthracis* should identify biomarkers to enhance product safety.

Division of Neurotoxicology Summary of Activities

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Introduction

Fifty-million Americans have a permanent neurological disability that limits their daily activities; one in three Americans will experience some form of mental disorder during their lifetime; and each year millions of children are exposed to anesthetics and sedatives that have been shown in pediatric-animal models to cause significant nerve-cell death. Health care, lost productivity, and other economic costs associated with brain-related diseases are estimated to exceed $500 billion annually. Disability from depression alone exceeds that of diabetes, hypertension, gastrointestinal, and lung diseases, costing over $43 billion annually. The number of persons with Alzheimer's and other age-related neurological disorders is increasing dramatically as our population ages. Known and suspected causes of brain-related disorders include exposures to chemicals, such as therapeutic drugs and drugs of abuse, food additives, food products, cosmetic ingredients, pesticides, and naturally occurring substances. Technological advances continue to provide new tools with which to better study and understand the causes of brain-related disorders, as well as the time-course and associated biological pathways involved with chemically-induced neurotoxicity, and to further reduce the risks associated with neurotoxic events.

The number of neuroactive chemicals that require FDA regulation is estimated to be in the thousands. Therefore, identifying methods to help pinpoint toxic mechanisms is critical for the development of safety guidelines and assessing neurotoxic risk. Chemicals that are known or suspected causes of brain-related disorders are vital to the national economy and our quality-of-life, so the challenge is to determine at what doses or exposure levels, and under what conditions, these compounds can be used effectively while minimizing the occurrence of adverse effects.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and identify biological pathways associated with the expression of neurotoxicity. Specific focus areas of fundamental research are employed to broadly examine the involvement of specific systems in the expression of neurotoxicity. These include:

- Monoamine-neurotransmitter systems.

- Mitochondrial function and oxidative stress.
- The N-methyl-D-aspartic acid (NMDA) and Gamma Amino Butyric Acid (GABA) receptor complexes and associated glutamate metabolism.
- The role of amyloid ß-peptide aggregation.
- The role of the blood-brain barrier.

An increased understanding of the processes associated with neurotoxic outcomes will provide opportunities for improved assessments of risk and identification of potential therapeutic approaches. The strategies employed for achieving these goals involve multidisciplinary approaches that capitalize on the neurochemistry, molecular neurobiology, neuropathology, neurophysiology, behavioral, and imaging expertise of Division personnel. In addition, efforts to develop sensitive, high-throughput systems for screening potential neurotoxicants have been developed (zebrafish) or are well underway (stem cells). Other unique features of the Division's research capabilities include the ability to:

- Determine chemical concentrations and cellular-level interactions in target tissue.
- Determine changes in gene- and protein-expression associated with chemical exposures.
- Effect high-throughput, comprehensive cognitive and behavioral assessments.
- Employ multiple species from zebrafish to rodent to nonhuman primates and—in some cases—to humans, in the risk-assessment process to increase confidence in extrapolating findings across species.
- Develop novel histochemical tracers to aid in the evaluation of chemical-induced pathologies.

FY 2011 Accomplishments

In partnership with FDA's Center for Drug Evaluation and Research (CDER) colleagues, Division staff continued to broaden their study of the neurotoxicity associated with pediatric anesthetics utilizing both *in vitro* (rodent and nonhuman primate neuronal-cell cultures) and *in vivo* (rat and nonhuman primate) approaches to include inhalation anesthetics. The data provided are vitally important for the regulatory needs of the agency and, further, this approach is beginning to identify compounds that may prevent or ameliorate anesthetic-induced neurotoxicity. Attempts to identify translatable biomarkers of nerve-cell death using Positron Emission Tomography (PET) technology have yielded a *Candida*te indicator of brain inflammation and additional assessments are continuing. Division scientists also continued their work with CDER reviewers to assist in the development of guidelines for the assessment of developmental neurotoxicity, neuropathology, and seizures.

Magnetic Resonance Imaging (MRI) approaches are now being applied to assess brain damage in rats after exposure to a variety of prototypic neurotoxicants with a view toward identifying ways in which imaging technology can inform the neurotoxicity risk-assessment process. Three-dimensional MRI data will be used to direct subsequent traditional neuropathological assessments as the first steps toward validating this approach.

Rodent studies on the neurodevelopmental toxicity of the ubiquitous plasticizer, bisphenol A (BPA), neared completion and data analyses are underway. In addition, data on the transplacental transfer of BPA were obtained from a nonhuman-primate model. These data will be important for assessing fetal exposure to BPA after maternal ingestion.

In collaboration with Wright-Patterson Air Force Base (WPAFB) and FDA colleagues at CDER and the Center for Devices and Radiological Health (CDRH), Division staff continued to characterize the adverse effects of a variety of nanomaterials using *in vitro* systems to model nerve cells, the blood-brain barrier, and associated microvasculature. Materials studied included carbon nanotubes, silver nanoparticles, and gold nanoparticles. It is clear that under some conditions these materials can produce oxidative stress, inflammation, and other toxic responses.

Utilizing a rat model of peripheral neuropathy induced by a mitochondrial toxin, it was shown that resveratrol is a potential *Candida*te for the treatment of metabolic neuropathy.

Ongoing studies on the neurobehavioral effects associated with adolescent methylphenidate (MPH) exposure in nonhuman primates indicate that, at serum levels matching or near human therapeutic levels, there are few detectable adverse effects. Given the widespread use of MPH to treat Attention Deficit and Hyperactivity Disorder (ADHD), these studies are providing important information about the safety of its chronic administration.

MicroPET imaging provided additional data on the time-course of ketamine-induced cell death in our rodent model. In addition, a new ligand for the identification of neuroinflammation (labeling of activated microglia/astrocytes) has shown to be effective in both the rodent and primate models of pediatric anesthetic-induced neurotoxicity. These imaging studies are critical to our efforts to identify radioligands appropriate for clinical use, thereby providing translatable biomarkers.

Studies on the assessment of human brain/cognitive function using the NCTR Operant Test Battery—the same instrument used in the Division of Neurotoxicity's Nonhuman Primate Research Center—continued at our laboratory at nearby Arkansas Children's Hospital (ACH), primarily in children with ADHD, depression, or anxiety disorder. A study to explore the long-term effects of ketamine in a pediatric population that directly parallels the work conducted in nonhuman primates at NCTR continues. Such studies

are exemplary of translational neuroscience and highlight the cross-species comparison capabilities within the Division.

FY 2012 Plans

Much of the work in FY 2012 will involve continuation of the efforts mentioned above, focusing on specific agency regulatory needs. These include:

- Publication of data from studies on bisphenol A, pediatric anesthetics, nanoparticles, antimicrobial peptides, and related compounds.
- Data on the efficacy and toxicity of a variety of potential therapeutic agents in a transgenic-mouse model of Alzheimer's disease will be submitted for publication.
- Renovations to expand the capacity of the Nonhuman Primate Research Center will continue. Utilization of the microPET, CT, and MRI imaging instruments will be expanded.
- Data on the transplacental transfer of BPA in the near-term rhesus macaques will be available and should provide a foundation for subsequent estimation of the potential exposure of the developing fetus to this agent after maternal exposure.
- Further utilization of the zebrafish developmental-neurotoxicity model should provide additional proof-of-concept data concerning species relevance.
- Utilization of our state-of-the-art imaging capabilities will provide new insights into the events contributing to adverse neural events.
- New histochemical tracers will be used to localize various elements of the brain vasculature and to illuminate the effects of neurotoxicants.
- In collaboration with WPAFB and FDA colleagues at CDER and CDRH, Division staff will continue to study the effects of nanomaterials on the integrity of the blood-brain barrier *in vitro* and *in vivo*.
- Novel MRI approaches will be utilized in regulatory science projects to develop standards for neuropathology protocols: MRI signals suggesting neurotoxicity will be used to direct follow-up traditional neuropathological assessments.
- As part of our ongoing validation process, studies at the NCTR laboratory, located at Arkansas Children's Hospital Laboratory, will continue to explore the relationship between the tasks that comprise the NCTR Operant Test Battery and standard psychological tests that are used clinically.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Neurotoxicology contributes to FDA Strategic Goal 3.1 (*Advance Food Safety and Nutrition*), Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*), and Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*). Relevant research is being conducted in support of NCTR Strategic Goal 1 (*Advance Scientific Approaches and Tools Necessary to Support Public Health*) under the following NCTR objectives:

Objective 1.1: Integrated Product Assessment
Research to elucidate the mechanisms underlying the neurotoxicity associated with the pediatric use of anesthetic agents include efforts in multiple *in vitro* and *in vivo* systems to define sensitive periods of development, explore critical dose-response relationships, and develop protective therapeutic strategies. Data from these efforts are continually provided to the agency and clinicians so they have the latest knowledge needed to minimize risk and protect children's health. Other studies employing exposures early in development (e.g., BPA) and in juvenile animal models (methylphenidate) are also providing data relevant to the pediatric use of these agents.

To assist the agency with the rapid determination of the neurotoxicity of a vast array of regulated chemicals and food contaminants, the Division has begun using a high-throughput, *in vitro* (zebrafish) system. This approach to identifying potential vertebrate toxicants will have broad applicability and be relevant for a variety of life stages—from fertilization throughout development. Coupled with our primary, organotypic, and stem-cell culture efforts, this approach will help direct subsequent resource utilization in further defining the risks associated with the use of regulated products.

The establishment of state-of-the-art imaging capabilities is providing opportunities to monitor the onset of toxic responses and to delve further into their mechanisms and time-course. These imaging resources provide the capabilities to get maximal information from invaluable preclinical models while minimizing the number of animals needed. It is hoped that development of the new magnetic resonance imaging (MRI) techniques to guide follow-up histopathological analyses will improve the safety of new drugs by decreasing the incidence of false-negative findings. Not only do these and similar efforts serve to strengthen FDA's base of operations, they also strengthen the scientific foundation of FDA's regulatory mission and the science that supports product safety.

Research at our ACH laboratory contributes to this priority by identifying appropriate, translatable biomarkers of brain function and their validity for assessing toxicity of the various foods and drugs that are regulated by FDA.

NCTR Objective 1.2: Advance regulatory science through the development of new tools and approaches

By developing effective methods for elucidating the biochemical pathways that underlie the expression of toxicity, it should be possible to use those methods to assess the toxic or beneficial effects of new medical and nutritive products. Utilization of *in vitro* brain cell and stem-cell cultures, as well as the zebrafish model, in our studies on the toxicity of anesthetic compounds are providing new information for understanding toxic mechanisms and should provide insight into possible rescue or protective approaches. This approach to identifying potential vertebrate toxicants will have broad applicability and be relevant for a variety of life stages—from fertilization throughout development. This approach will help direct subsequent resource utilization in further defining the risks associated with the use of regulated products. A transgenic mouse model of Alzheimer's plaque deposition is being used in efforts to help delineate toxic mechanisms and explore the potential efficacy of different therapeutic strategies. The establishment of state-of-the-art imaging capabilities is providing opportunities to monitor the onset of toxic responses and to delve further into their mechanisms and time courses. These imaging resources provide the opportunity to obtain maximal information from invaluable preclinical models while minimizing the number of animals needed. It is hoped that our approach to using MRI techniques to guide follow-up histopathological analyses will improve the safety of new products by decreasing the incidence of false-negative findings. Not only do these and similar efforts serve to strengthen FDA's base of operations, they also strengthen the scientific foundation of FDA's regulatory mission and the science that supports product safety.

Many of these efforts involve partnerships within the agency, with industry, and with academic centers. In addition, Division staff continue to provide training for undergraduate and graduate students, postdoctoral fellows, visiting scientists, and participants in the FDA Commissioner's Fellowship Program, many of whom will go on to serve the agency as employees endowed with the knowledge and expertise needed to preserve its science base.

Division of Personalized Nutrition and Medicine Summary of Activities

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Introduction

The Division of Personalized Nutrition and Medicine is charged with understanding how individual genotypes interact with environmental factors to influence individual health. Recent data has confirmed that while humans are genetically similar, each retains a unique genetic identity that contributes to the wide array of biochemical, physiological, and morphological phenotypes in human populations. Parallel molecular genetic studies have demonstrated that nutrient and environmental chemicals directly or indirectly regulate the expression of one's genetic makeup.

While the research strategies of the 20th century yielded data and knowledge that extended our average lifespan and improved personal and public health, much of that knowledge was based on the average response of a population to a nutrient, pharmaceutical agent or environmental chemical, or the average risk for carrying a specific allele of a gene involved in disease. Such knowledge may or may not be applicable to an individual with different genotypes or environmental exposures.

The overall goals of the Division are to develop and implement research strategies that account for genetic and environmental diversity that influence expression of genotypes and produce knowledge for improving personal and public health. These overarching goals will be met with three parallel efforts that develop:

- Integration of omics methodologies to assess an individual's health status and, as importantly, susceptibility to specific chronic conditions influenced by environmental factors, including diet.

- Classification algorithms that integrate data from omics and environmental assessments that will result in evidence-based and validated biomedical-decision making.

- A novel pathogen knowledge base for the Food Protection Plan that will become the foundation for a metagenomic (human microbiota) program within the Division.

The Division has two areas—Biometry and Biology. The main function of the Biometry area is to develop biometrical methods for all aspects of FDA's mission, goals, and objectives. A subgroup within the area analyzes all data from the National Toxicology

Program (NTP). The Biology area is focusing on the broad areas of pharmacogenomics and nutrigenomics—how individuals respond to drugs and nutrients in foods.

FY 2011 Accomplishments

The Division of Personalized Nutrition and Medicine met major milestones in FY 2011 and laid the foundation for future programs in FY 2012 and beyond.

The National Toxicology Program (NTP) statistician subgroup of the Biometry program completed 63 statistical reports for NTP protocols. Additionally, the group has completed 30 analyses for the NCTR bisphenol-A (BPA) neurotoxicity study, and performed randomization for four NTP and two non-NTP studies. Members of this team also provided statistical support to other protocols, including protocol review for a number of additional NTP and non-NTP studies and reviewed protocols for the Institutional Animal Care and Use Committee (IACUC).

The statisticians in the Biometry area continue to contribute to multiple Division of Personalized Nutrition and Medicine projects, NCTR projects, and FDA Product Center projects, and continue to maintain communications with the scientists on risk-assessment methodology in the Interagency Risk-Assessment Consortium (IRAC). The research efforts focused on statistical and data-mining methods for the analyses of high-dimensional genomic, proteomic, metabolomic, and toxicoinformatic data. Projects related to microarray data analysis include:

- Sample-size estimation for high-dimensional data.
- Gene-set enrichment analysis methodology.
- Survival risk prediction for treatment decision.
- Association analysis in drug susceptibility.
- Biomarkers for ethnic differences in breast cancer.

Projects related to data mining and bioinformatics include:

- Feature selection and biomarker identification.
- Ensemble classification algorithms.
- Imbalanced class-size prediction.
- Bicluster analysis algorithms.
- Biomarker classifiers for identifying susceptible subpopulations.
- Genomics knowledge base for detection and characterization of microbiological pathogens.

These efforts are leading the way to an improved ability to develop classification algorithms. Algorithms, or a precise rule (or set of rules) specifying how to solve problems, contribute to the ease of use of high-dimensional genomic biomarkers, and

thus contribute to the development of statistical methods to analyze individual genes and biological pathways, QT analysis and cardiotoxicity, and investigate hierarchical-probabilistic models for categorization of uncertainty in risk/safety assessment.

The Biometry area is also leading a cross-division program to develop a food-pathogen knowledge base as part of FDA's Food Protection Plan. Division researchers and statisticians published a collaborative study testing new algorithms to assess relatedness of the foodborne pathogen, *Salmonella*.

Division scientists from both the Biology and Biometry areas were involved in a community-based participatory research program to examine how providing children with nutritious foods affected blood-vitamin concentrations and to examine the role that individual genotypes might play in affecting the blood-vitamin levels. Samples were collected in 2009 and 2010, and data analysis is nearly complete. Novel statistical analyses are being used that will eventually enable the analyses of gene-gene interactions that could provide insight into individual responses to drugs, nutrients, and toxicants.

Division scientists from the Biology program are also collaborating on studies, which examine associations between genetic make-up and various disease states, as well as responses to drugs. This area of pharmacogenetics is an area that will continue to grow in importance as the Center works toward assisting FDA in the future of personalized medicine.

The Division's stem-cell program is examining the effects of toxins on development, differentiation, and the influence of nutrients on metabolism. Division scientists are developing the mouse Embryonic Stem Cell Test, a test that predicts developmental toxicity without the use of any laboratory animals. The test is being expanded to include additional, more objective, and quantifiable endpoints. The test is also being expanded to include differentiation to additional cell lineages in an attempt to improve the predictivity of the test.

The ability of human mesenchymal stem cells to differentiate to adipocytes is being used as a novel system to investigate toxicity. Initial studies have examined only differentiation to adipocytes, but these stem cells are also able to differentiate to other cell lineages, and future work will focus on the other cell lineages. Other stem-cell work focuses on nutrient metabolism and effects of various nutrients on normal metabolic pathways. Our long-term goal is to integrate data and results from the model systems of stem cells, laboratory animals, and humans to provide results, which range from mechanisms to applications in humans.

FY 2012 Plans

Division of Personalized Nutrition and Medicine investigators will conduct the following research studies in FY 2012.

- The use of alternative tests for developmental toxicity in regulatory science is being promoted by industry, and the ability of these tests to adequately predict toxicity is an unanswered question. Members of the Division are expanding the mouse Embryonic Stem Cell Test to determine its ability to predict developmental toxicity. The assay is being expanded beyond the current validated test by including additional endpoints and differentiation to additional cell lineages.

- Division scientists are also developing a protocol in collaboration with scientists from the Division of Neurotoxicology to compare the predictivity of developmental toxicity testing using the mouse Embryonic Stem Cell Test or a zebrafish assay.

- The effects of various compounds on the differentiation of human mesenchymal stem cells to adipocytes will continue to be examined. Additionally, these stem cells will be differentiated to other cell types to determine the effects of toxicants on differentiation to multiple cell lineages.

- The effects of nutrient metabolism on human stem cells remains an ongoing investigation. Preliminary data show that fructose alters glucose metabolism in adipocytes in culture. These results add to a growing body of literature indicating that intake of high levels of fructose may be detrimental to health. This work will be extended to include additional cell lines, as well as a study using a whole-animal model system.

- Division scientists continue to collaborate with scientists from the United States as well as international scientists on the identification of genetic factors which either increase an individual's susceptibility to the adverse effects of a drug or increase the efficacy of a drug for a particular subpopulation.

- The Biometry program will continue to investigate methods for integrating the associations between the genomic-predictor variables and phenotype-class variables (such as tumor types or treatment efficacy), predictive models, and computational methods for quantitative assessment of benefit/risk models for regulatory decisions in personalized medicine.

- Initiate research on biostatistical approach for relative-risk ranking for food protection.

- Biometry scientists are developing decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes. A prediction model will be developed to select patients to receive or to avoid a

drug/treatment to improve (reduce) the benefit (risk) of the drug/treatment. Experimental designs and data-analysis strategies for evaluation of studies for drug-device co-developed products will be developed.

- Biometry scientists are developing integrated analysis of single-nucleotide polymorphisms and copy-number variation to assess the association between the genetic variation and breast-cancer status in different ethnic groups.

- Biometry scientists are developing methods to assess cardiac safety of drugs. Statistical modeling and procedures are being developed to obtain accurate measurements of QT interval correction to identify true QT prolongation.

- Biometry scientists are developing a research protocol to investigate genetic and genomic diversity of *Listeria monocytogenes* in pregnancy-associated *Listeriosis*.

- Biometry scientists will continue to lead the research project for an integrated genomics knowledge base for rapid threat assessment of enteric foodborne pathogens. This project is in collaboration with the Divisions of Microbiology and Systems Biology.

- The Biometry program will continue to develop computation algorithms:
    a. Data-mining algorithms and matrix visualization to identify local structures in high-dimensional data matrix for applications to the Adverse Event Reporting System database to identify which sets of drugs are associated with which sets of adverse events.
    b. Computational software to compute p-values and adjusted p-values for gene-set enrichment analysis defined through pathways and gene ontology.
    c. Network algorithms to identify genomic, proteomic, and metabolomic liver-toxicity biomarkers.

- The NTP staff will continue its critical mission of analyzing data from NTP studies.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Personalized Nutrition and Medicine contributes to FDA Strategic Goal 3.1 (*Advance Food Safety and Nutrition*), Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*), and Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*). Relevant research is being conducted in support of NCTR Strategic Goal 1 (Advance Scientific Approaches and Tools Necessary to Support Public Health).

Division members contribute by helping to identify and evaluate biomarkers and endpoints that can be used in nonclinical and clinical evaluations. Scientists are: 1) evaluating the accuracy (specificity and sensitivity) with which animal models and cell-based assays correctly predict human; and 2) assessing the concordance between animal and human markers of toxicity and determining how the performance of these markers and their interpretation may vary across different organ systems and human populations. Examples include the continued development of the mouse Embryonic Stem Cell Test and expansion to include human embryonic stem-cell lines; this test has been suggested to replace a portion of the preclinical testing for developmental toxicology in animal species.

The Division's Biometry Group is helping to develop and refine clinical trial designs, endpoints,and analysis methods. Scientists are: 1) continuing to refine clinical trial design and statistical methods of analysis to address issues such as missing data, multiple endpoints, patient enrichment, and adaptive designs; 2) continuing to refine the use of modeling and simulation in clinical trial design to enhance the effectiveness of clinical studies; and 3) continuing development and refinement of tools and approaches for assessing benefit/risk. Examples include developing decision models for clinical assignments of patients based on the patient's genomic features and disease phenotypes, analysis of a number of high-dimensional data sets, QT prolongation and cardiotoxicity studies, as well as the development of a knowledge base for rapid threat assessment of enteric foodborne pathogens.

Division members are also working to increase the accuracy and consistency, and reduce inter-platform variability of analytical methods to measure biomarkers by continuing to participate in collaborative efforts, such as the MicroArray Quality Control Consortium, to evaluate the quality of validation strategies for emerging technologies.

Overall, the Division of Personalized Nutrition and Medicine provides expert advice and innovative research to the other FDA Centers—thus contributing to FDA's overall mission of advancing public health. Research projects involve new and innovative technologies and approaches that support several of FDA's regulatory Centers.

Division of Systems Biology Summary of Activities

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Introduction

The Division of Systems Biology supports the development of new technologies and identification of new biomarkers to facilitate the integration of scientific data for application to questions that are in direct support of the FDA regulatory mission. Six Centers of Excellence comprise the Division of Systems Biology:

- Bioinformatics
- Functional Genomics
- Hepatotoxicity
- Innovative Technologies
- Metabolomics
- Proteomics

This multidisciplinary group is comprised of microbiologists, biologists, analytical chemists, applied statisticians, physical chemists, physicists, bioinformaticians, etc. The goals of this Division are to convert emerging science and technology into:

1) Finding new translational biomarkers to improve detection of drug and food-supplement safety issues, disease onset, and progression.
2) Developing innovative tools to improve detection of food contaminants, identification of infectious diseases, and diagnostic procedures.
3) Building bioinformatics solutions that facilitate the regulatory process.

The **Center for Bioinformatics** conducts research in bioinformatics and chemoinformatics and develops and coordinates informatics capabilities within NCTR, across FDA Product Centers, and in the larger scientific community. A goal of this group is to develop methods for the analysis and integration of omics (genomics, proteomics, and metabolomics) datasets with classical in-life parameters. This group is taking an active role in supporting bioinformatics needs of other FDA centers and is active in reviewing Voluntary Exploratory Data Submissions (VXDS) submitted to FDA's Center for Drug Evaluation and Research.

The **Center for Functional Genomics** uses high-information content microarrays to identify biomarkers for improved safety assessments and disease management. Major efforts include: developing preclinical predictive-toxicology biomarkers, understanding the mechanistic links between mitochondrial dysfunction with toxicity and disease processes, and continuing to serve as an FDA resource for genomic issues.

The **Center for Hepatotoxicity** addresses critical issues related to liver injury by applying a systems-biology approach to find biomarkers. The goals include the identification of biomarkers that will improve the preclinical identification of drugs and dietary supplements that prove to be hepatotoxic in humans and to augment the detection of early signs of liver injury in humans induced by drugs, chemicals, and disease processes.

The **Center for Innovative Technologies** uses multifaceted approaches to address important issues of human health. Examples include programs in mass spectrometry- and flow cytometric-based analyses for rapid detection of bacteria in food and clinical samples and significant computational efforts in modeling to improve diagnosis and toxicity-risk assessment.

The **Center for Metabolomics** employs open, focused, and flux-based metabolomic analytical profiles to improve the detection of toxicity and disease in preclinical and human studies. The group is focused in connecting the results of these metabolomics studies to magnetic resonance spectroscopy imaging (MRI) studies

The **Center for Proteomics** conducts proteomic research to address FDA critical issues related to drug safety and efficacy, and early disease detection. The Center continues to develop and evaluate novel proteomic technologies with the aim of facilitating the translation of basic science to medical products.

FY 2011 Accomplishments

During FY 2011, Division scientists engaged in research addressing a variety of agency issues with special emphasis on areas in biomarker identification, food safety, improvement of diagnostic procedures, and bioinformatics. Accomplishments include the following:

Biomarkers of Tissue Injury

1) Nephrotoxicity (kidney)
   1) Several years ago, dogs and cats developed kidney damage following unintentional ingestion of food containing melamine and cyanuric acid. A metabolomics-based approach was used in a rat model to discover potential biomarkers of this injury.
   2) Drug-induced nephrotoxicity continues to be a problem in drug development and clinical care and new biomarkers are needed. A metabolomics approach was

used in collaboration with investigators at CDER to investigate gentamicin-induced nephrotoxicity.

3) Aristolochic acid, a dietary supplement, has been found to cause kidney damage in humans and animals. Using a proteomics approach, potential biomarkers of renal injury in the rat were identified.

4) Age- and gender-based differences in response to medicines and disease susceptibility continue to be reported in humans. To identify a potential role of genes in this phenomenon, a genomics approach was used in aging rats of both sexes.

2) Hepatotoxicity (liver)

- Usnic acid is a dietary supplement shown to induce hepatotoxicity in some humans. Metabolomics approaches were used on *in vitro* studies done with hepatocytes exposed to usnic acid. The results supported a mechanistic understanding of how this dietary supplement induces hepatotoxicity in humans.
- New biomarkers of liver damage are needed to improve detection of this injury in animals and humans. A genomics approach was used on urine samples of rats treated with drugs and chemicals that cause liver injury. Several urinary miRNAs (also called epigenetic biomarkers) were identified that may serve as predictive biomarkers of hepatotoxicity. Using a combined genomics and metabolomics approach, Division scientists identified new blood biomarkers of drug- and chemical-induced liver injury.
- As we age, Americans are taking more drugs (prescription and over-the-counter) and dietary supplements (polypharmacy). This raises a concern about how these agents might work together and cause organ injury. Acetaminophen is a widely used over-the-counter drug that can cause severe and sometimes fatal liver injury. This study was conducted to investigate if popular dietary supplements might exacerbate the potential of acetaminophen to cause hepatotoxicity. Using an *in vitro* approach, some dietary supplements have been found to increase the hepatotoxicity of acetaminophen. *In vitro* studies are being conducted to identify the mechanism of this effect.

3) Cardiotoxicity (heart)

- Doxorubicin is an important chemotherapeutic drug that can cause heart damage in humans. Early warning signs of such injury are needed so that drug therapy can be discontinued. To identify new biomarkers of such injury, a mouse model of doxorubicin-induced cardiotoxicity was developed.
- A study is being conducted to evaluate the potential cardiotoxicity of chronic methylphenidate exposure.

Bioinformatics Approaches

- The amount of data present in the public domain and generated at NCTR is very difficult to interpret manually. Thus, bioinformatics approaches are being applied to identify connections that can be used for hypothesis-based research. One such project is the development of a liver toxicity knowledge base (LTKB). The goal is to collect all clinical parameters of each drug that has been reported to cause liver injury and to generate gene-expression data from *in vitro* studies and other studies to identify potential biomarkers. This work continues.
- Another project involves leading an international consortium that is studying a new approach to identify gene-expression alterations in human and rat tissues. This next-generation sequencing approach generates very large datasets that challenges analysis approaches. This consortium is looking at appropriate approaches and standards to enable use of this developing science.
- Supporting the analysis of gene-expression results has been a standard function of this bioinformatics group. They developed a software package that is used within and outside FDA called ArrayTrack™™. Improvements are being made to ArrayTrack™™ to assist the VXDS process.

This group continues to support CDER efforts and this includes developing databases for their use.

Technology Development/Improvement

- Developed new analytical methods to identify potential biomarkers of tissue injury in the blood and urine.
- Designed and began preliminary testing of mitochondria-specific gene-expression arrays for mouse and rat on a commercial platform. This led to better safety evaluation of drugs that may cause tissue injury by targeting mitochondria.
- In collaboration with CDRH, completed studies to characterize whole genome amplified DNA for its suitability for genetic-device validation.
- Developed novel proteomic sample preparation techniques for the identification of a new class of potential proteomic biomarkers (e.g., N-terminal peptides) using mass spectrometry.
- Improved mass-spectrometry techniques for the identification of protein modifications, such as phosphorylation, to expand the range of biomarker discovery.

Personalized Medicine

- Leflunomide is a drug that is known to cause liver injury in a small percentage of people. If the susceptibility factors could be discovered, it might lead to safer use of this drug. Studies with *in vitro* cells suggest that variations in the way this drug

is broken down by individuals may explain the difference in susceptibility. This could lead to a diagnostic test that could inform personalized medicine.

- Reports of drugs directly interacting with some individual's unique genetic sequences have been reported to be the basis for adverse drug reactions. If this can be modeled via computer (*in silico*), it could improve drug development and assessment of individuals' risk to particular drugs. This area is challenging as the structural information of all genetic variants are not known. However, progress continues to be made.

Food Safety

- Work continues on efforts to improve the testing of food for bacterial contamination. Technical approaches were developed to remove background interference from food types that tend to be difficult to analyze by existing methods. Examples include milk, peanut butter, chocolate, and raw cookie dough. New assays were developed for additional bacteria known to cause human-health concerns when found in food and when present in human clinical samples.

Safety of Nanoparticles

- A proteomics approach was used to identify potential protein biomarkers of early response to titanium dioxide exposure

FY 2012 Plans

In FY 2012, the Division of Systems Biology will continue to emphasize a systems-biology approach for development of biomarkers and mechanistic information for safety and efficacy assessments of medical products and foods. Additional studies will focus on the development of improved *in silico* modeling approaches for drug and food-supplement safety and medical imaging. New studies will begin to discover biomarkers of tobacco-related injury. To accomplish its mission, the Division of Systems Biology will continue to study: 1) toxicity, 2) efficacy, and 3) disease-utilizing systems-biological approaches, and 4) other methods to identify new biomarkers that will improve the safety, efficacy, review, and usefulness of FDA-regulated products.

- Identify potential biomarkers to improve early detection of idiosyncratic hepatotoxicants and to explore dietary-supplement interaction with acetaminophen-induced hepatotoxicity in a multi-year, systems-biology study.
- Identify biomarkers of drug-induced cardiotoxicity.
- Begin connecting systems-biology data in preclinical studies with imaging biomarkers.
- Establish targeted proteomic analysis pipelines to accelerate biomarker discovery.

- Maintain efforts to build data repositories and improve *in silico* methods and analysis tools.
- Expand the Liver Toxicity Knowledge Base.

The Division will also continue to:

- Conduct studies on age- and sex-related gene-expression profiles in rat organs and relate findings to potential age- and sex-related susceptibilities to drug toxicities and effectiveness in the rat model, as well as in humans.
- Develop open profiling and focused metabolomic analyses of biofluids and tissues from preclinical and clinical studies and utilize flux analyses to identify early biomarkers of tissue injury or dysfunction.
- Develop intra-lab and inter-lab quality-control standards for metabolomics analyses.
- Use proteomic approaches to identify biomarkers and mechanisms of diseases and drug-induced organ toxicity, including liver, kidney, and heart.
- Conduct studies to improve food safety and infectious disease diagnosis.
- Evaluate *in silico* technology related to protein-drug interaction for personalized medicine.
- Expand ArrayTrack™™ to warehouse, visualize, analyze, and interpret data from diverse omics technologies, as well as clinical and nonclinical data.
- Support FDA's regulatory mission.
- Provide technical expertise to FDA in genomic, proteomic, and metabolomic interpretation and guidance.
- Evaluate the technical performance and practical utility of next-generation sequencing technology via the MAQC-III (SEQC) project.
- Participate in the review of the pharmacogenomics data submitted through the Voluntary Exploratory Data Submission (VXDS) program.

Contributions to FDA's Strategic Priorities/Goals

The research conducted by the Division of Systems Biology contributes to NCTR Strategic Goal 1 (*Advance Scientific Approaches and Tools Necessary To Support Public Health*) and to FDA Strategic Goal 3.1 (*Advance Food Safety and Nutrition*), Goal 3.2 (*Promote Public Health by Advancing the Safety and Effectiveness of Medical Products*), and Goal 2.1 (*Cross-Cutting Research to Advance Regulatory Science and Innovation*).

The Division provides expert advice and innovative research to the other FDA Centers,

thus contributing to FDA's mission of advancing public health. Research projects involve new and innovative technologies and approaches that support FDA's regulatory Centers.

The Division of Systems Biology will continue to utilize multifaceted approaches (including genomic, proteomic, and metabolomic tools) to discover predictive and diagnostic biomarkers to improve drug safety and efficacy and disease prevention and management. New studies are beginning to identify biomarkers of deleterious effects of tobacco use *in vitro*. Efforts will continue to be made to utilize *in silico* (computer simulated) approaches for predictive toxicology to enhance product safety. Continued development of RAPID-B™ will support food and biological products.

The MAQC-III consortium aims to determine and identify the issues and challenges associated with the next generation of sequencing technology. It anticipates that the review of such data as a part of an Investigational New Drug (IND) and New Drug Application (NDA) submission will soon become an FDA responsibility.

Innovation will be continue to be a hallmark of this Division and will include the development of new proteomic methods to enable discovery of new types of biomarkers, the merging of metabolomics and bio-imaging to find translational biomarkers, and improvements in mass-spectrometric technologies. The continued development of new bioinformatics tools will allow reviewers to easily access information from both private and public domain—thus enhancing the FDA review process. Novel computational models will continue to be developed that predict drug safety and efficacy. These new methods will increase the number of safe and effective medical products.

PI: Ahn, Young-Beom, Ph.D.

Exploring Strategies for Resuscitation and Enrichment of *Burkholderia Cepacia* Complex Strains in Pharmaceutical Products (E0749801)

Responsible Division: Microbiology
Collaborating FDA Center: CDER
Objectives:

1) Screen and identify strains of *B. cepacia* that are difficult to cultivate from pharmaceutical water.
2) Develop a resuscitative step and enrichment technique for *B. cepacia* complex recovery.
3) Develop methodology to detect *B. cepacia* and its 16 related genomovars.
4) Evaluate the use of modern molecular technologies to identify B. cepacia complex.

PI: Ahn, Young-Beom, Ph.D.

Impact of Antimicrobial Residues on the Human Gastrointestinal Tract Microbiota (E0732701)

Responsible Division: Microbiology
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Collaborating FDA Center: CVM
Objectives:

1) Develop methodology to determine if antimicrobial-agent residues bound to fecal contents are microbiologically active.
2) Evaluate the use of current molecular biology, genomic, and proteomic technologies to determine the impact of antimicrobial-agent residues on the human-intestinal microbiota.
3) Determine the potential of the intestinal microbiota to metabolize antimicrobial residues.

PI: Ali, Syed F., Ph.D.

DNA Neurotoxicity Assessment of Manganese (Mn)-Nanoparticles in PC-12 Cells and in Mice (E0725701)

Responsible Division: Neurotoxicology
Objectives:

1) Evaluate the neurotoxicity of different-sized Mn-nanoparticles using PC-12 cultured cells.
2) Determine if *in vitro* exposure to Mn-nanoparticles selectively induces specific genomic changes in PC-12 cultured cells using oligonucleotide microarrays.
3) Determine if multiple doses of Mn-nanoparticles produce reactive oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase), and levels of glutathione in various regions of the mouse brain.
4) Determine if single or multiple doses of Mn-nanoparticles induce specific genomic changes in various regions of the mouse brain using oligonucleotide microarrays.
5) Determine if single or multiple doses of Mn-nanoparticles produce significant changes in neurotransmitter concentrations in various regions of the mouse brain.
6) Determine if single or multiple doses of Mn-nanoparticles produce significant changes in the formation of 3-nitrotyrosine, an *in vivo*

biomarker for oxidative stress, in various regions of the mouse brain.
7) Determine if multiple doses of Mn-nanoparticles produce morphological alterations in the brain or visceral organs of the mouse.

PI: Ali, Syed F., Ph.D.

Neurotoxicity Assessment of Cell Phone Radio-Frequency Radiation Using Rat and Bovine Brain Microvascular-Endothelial Cells as Model Blood Brain Barrier Systems, PC-12 Cultured Cells, and Whole-Animal (Mice and Rats) Models (E0217301)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDRH
External Partner: National Toxicology Program
Objective:
Determine whether power levels of radiofrequency radiation that are emitted from mobile phones produce any changes in the central nervous system of mice and rats.

PI: Ali, Syed F., Ph.D.

Neurotoxicity Assessment of Silver (Ag)-Nanoparticles in PC-12 Cells and in Rats (E0728201)

Responsible Division: Neurotoxicology
Collaborating Division/Office:
Personalized Nutrition and Medicine, Office of Scientific Coordination
Objectives:
1) Evaluate the neurotoxicity of different sizes of Ag-nanoparticles using cultured PC-12 cells.
2) Determine if *in vitro* exposure to Ag-nanoparticles selectively induces specific genomic changes in cultured PC-12 cells using microarrays.
3) Determine if single or multiple doses of Ag-nanoparticles produce reactive oxygen species, alterations in lipid peroxidation and/or changes in antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) and glutathione levels in the rat brain.
4) Determine if single or multiple doses of Ag-nanoparticles induce specific genomic changes in the rat brain as indicated with microarrays.
5) Determine if single or multiple doses of Ag-nanoparticles produce significant changes in neurotransmitter concentrations in the rat brain.
6) Determine if single or multiple doses of Ag-nanoparticles produce significant changes in the formation of 3-nitrotyrosine (3-NT), an *in vivo* biomarker for oxidative stress, in the rat brain.
7) Determine if multiple doses of Ag-nanoparticles produce morphological alterations in blood-brain barrier, brain, or other visceral organs of the rat.

PI: Azevedo, Marli, Ph.D.

Molecular and Seroepidemiology of Coronavirus and Disease Spectrum in Adults, Children, Domestic Animals, and Wildlife in the U.S. (E0738001)

Responsible Division: Microbiology
Collaborating FDA Center: CBER
Objectives:
1) Investigate the molecular epidemiology of circulating enteric and respiratory human CoV (HCoV) and nonhuman CoV strains.
2) Determine whether there is substantial genetic variability of HCoV in our community and examine geographical genetic

variation by comparisons with published findings.

3) Determine the zoonotic potential and health-safety threat of newly emerging CoV by comparing to strains currently circulating in domestic animals and wildlife.
4) Define the seroepidemiology and cross-reactivity of circulating HCoV with known human and nonhuman CoV.
5) Generate immunobiologicals to develop an immunoassay to detect HCoV anti bodies for new strains with high antigenic variation.
6) Use the HCoV-specific antibody ELISA or virus neutralization assays to define the seroepidemiology of the newly detected viruses in adults and children and estimate their prevalence.

PI: Beger, Richard, Ph.D.

3D- and 4D-QSDAR Modeling Applied to Various Toxicological Endpoints (E0734801)

Responsible Division: Systems Biology
Collaborating FDA Center: CDER
Objectives:

1) Develop 3D- and 4D-quantitative spectroscopic data-activity relationships (QSDAR) models for endocrine disruptors, lowest-observed-adverse-effects level, no observed-adverse-effects level, and other relevant toxicological endpoints.
2) Test the training models with external test sets.
3) Compare the training and testing results to previous QSDAR, quantitative structure-activity relationship, and structure activity relationship models.
4) Determine how the technique used to predict 13C or 15N nuclear magnetic resonance (NMR) spectra affects 3-D-QSDAR modeling. One technique will use ACD/Labs Predictor software, which references 2D descriptors for compound fragments and approximately 20 million experimental NMR chemical shifts. The other determines 13C and 15N NMR spectra from ab initio calculations of structural conformations for each compound.

PI: Beger, Richard, Ph.D.

Quality Control for Focused and Unfocused LC/MS-Based Metabolomic Profiling of Blood Samples (E0738401)

Responsible Division: Systems Biology
Objective:
Develop and test a quality control protocol on existing preclinical hepatotoxicity protocols that can potentially be translated to future clinical metabolomics experiments.

PI: Beland, Frederick A., Ph.D.

Benzocaine-Induced Methemoglobinemia in an Acute Rat Model (E0730201)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CVM
Objective:
Produce data that will provide information on the potential for benzocaine-induced methemoglobinemia in humans consuming meat from benzocaine-treated fish.

PI: Beland, Frederick A., Ph.D.

Mechanisms of Nevirapine Carcinogenicity (E0217101)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDER
External Partner: National Toxicology Program
Objective:
Determine the mechanisms by which nevirapine induces liver tumors in rats.

PI: Beland, Frederick A., Ph.D.

Perinatal Carcinogenicity of Drug Combinations Used to Prevent Mother-to-Child Transmission of HIV (E0214111)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicoogy, Systems Biology
Collaborating FDA Center: CDER
External Partner: National Toxicology Program
Objective:
Determine the carcinogenicity, genotoxicity, and metabolism of antiretroviral drug combinations administered to mice transplacentally, perinatally, or neonatally.

PI: Boudreau, Mary D., Ph.D.

13-Week Study To Evaluate the Toxicology of Silver (Ag)-Nanoscale Particles (NP) in Sprague-Dawley Rats. (E0218001)

Responsible Division: Biochemical Toxicology
Collaborating Divisions/Office: Genetic and Molecular Toxicology, Personalized Nutrition and Medicine, Office of Scientific Coordination
External Partner: National Toxicology Program
Objective:
Determine the toxicity of different sizes of Ag-NP when administered daily by gavage to Sprague-Dawley rats.

PI: Boudreau, Mary D., Ph.D.

A Toxicological Evaluation of Silver (Ag)-Nanoscale Particles (NP) in Rodents (E0217001)

Responsible Division: Biochemical Toxicology
Collaborating Divisions:
Neurotoxicology, Personalized Nutrition and Medicine
Objectives:

1) Evaluate the effect of size of Ag-NP on plasma protein-binding in blood collected from adult rodents using standard analysis methods to estimate the equilibrium association constant and maximum binding capacity.
2) Determine the effects of size and dose of Ag-NP on the pharmacokinetic profiles and bioavailability when administered by the oral and intravenous routes in rats, and determine whether the pharmacokinetics of Ag-NP are the same as Ag-acetate.
3) Evaluate the absorption, biodistribution (including the potential to cross the blood-brain barrier), and excretion rates of Ag-NP that differ in size.

PI: Boudreau, Mary D., Ph.D.

An Evaluation of the Effect of Vehicle Cream on the Photococarcinogenicity of Retinyl Palmitate in SKH-1 Mice (E0218501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN

Objectives:

1) Determine the stability and homogeneity of retinyl palmitate in the *Aloe vera* control cream.
2) Evaluate the photococarcinogenicity of retinyl palmitate when incorporated into the *Aloe vera* control cream applied to the skins of SKH-1 mice in the absence and presence of simulated solar light (SSL).
3) Determine the photococarcinogenicity disopropyl adipate as the filler ingredient in the *Aloe vera* control cream in the absence and presence of SSL.

PI: Boudreau, Mary D., Ph.D.

Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice (E0214301)

Responsible Division: Biochemical Toxicology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CFSAN
Objective:
Study the effects of topically applied skin cream containing retinyl palmitate on the photocarcinogenicity of simulated solar light in SKH-1 mice.

PI: Bowyer, John F., Ph.D.

Characterizing the Amphetamine (AMPH)-Induced Changes in Vascular Tone, Vasotrauma, and Alterations in Angiogenesis in Rodent Brain (E0729501)

Responsible Division: Neurotoxicology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CDER
Objectives:

1) Evaluate the effects of both acute and chronic AMPH exposure on the vasculature of the rat brain.
2) Examine vasculature within the parenchyma of three brain regions; the striatum, parietal cortex, and the combined piriform and amygdaloid nuclear cortices where AMPH-induced neurodegeneration can occur.
3) Determine the alterations in vascular gene expression after different periods of exposure.

PI: Bowyer, John, F., Ph.D.

Studies Comparing the Neurotoxicology of Amphetamine (AMPH) with Methamphetamine (METH) and Methylphenidate (MP) (E0740101)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine
Collaborating FDA Center: CDER
Objectives:

1) Determine the appropriate dose range and plasma levels of MP that produce a hyperthermic profile similar to that produced by neurotoxic doses of AMPH and METH. Then, using that dose range, compare the neurotoxicity produced by MP with that of AMPH and METH.
2) Evaluate the hyperthermic profiles resulting from the selected doses of MP, AMPH, or METH, and their relationship between the hyperthermic profile and neurotoxic outcome of these compounds.
3) Determine the effects of non-neurotoxic doses of MP, AMPH, or METH on body temperature and

gene expression, which is to be compared to neurotoxic doses.

4) Determine whether a single, very high dose of either AMPH or METH—which produces seizure activity—has substantially different effects on body temperature than the multiple "lower" dose neurotoxic exposures (four consecutive doses in the 3-10mg/kg range) that do not produce seizures.

PI: Cao, Xuefei, Ph.D.

Dose-Response Genotoxicity of Ethylmethane Sulfonate (EMS) in Mice Using the Pig-a and Transgenic gpt Delta Assays (E0739001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CDER
Objectives:

1) Use sensitive genotoxicity endpoints with low background frequencies to increase the sensitivity of the assays for detecting low-dose effects.
2) Measure genotoxicity using a design to detect the maximum responses.
3) Measure the effects of EMS exposure in neonatal as well as adult animals.
4) Measure genotoxicity in the major target tissues for EMS carcinogenicity.

PI: Chelonis, John, Ph.D.

ASK CHILDREN Study — Assess Specific Kinds of Children Challenges for Neurologic Devices (E0734301)

Responsible Office: Neurotoxicology
Objectives:

1) Establish a science-based framework of recommendations to help develop more efficient strategies in evaluating pediatric products regulated by FDA.
2) Develop a framework of science-based recommendations important to help expedite pediatric prostheses to market, including recommendations for the research and development of neurologic devices.
3) Collect qualitative and quantitative self-report clinical data (through interviews) and identify scientific and medical issues associated with pediatric devices when used in children undergoing treatment, to develop more efficient strategies for evaluating these types of products regulated by FDA.
4) Organize data that are important to developing more efficient strategies in evaluating these types of products regulated by FDA into multiple categories, including (but not limited to); device type, pediatric subpopulations, disorder or condition, and intended use.

PI: Chelonis, John, Ph.D.

Development and Validation of Interspecies Cognitive Assessments (E0735501)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Research
Objective:
Compare children's performance on operant tests that have been used extensively to assess drug effects in animals with performance on neuropsychological tests that are typically used in clinical settings that are thought to measure similar cognitive functions.

PI: Chelonis, John, Ph.D.

Long-Term Neurodevelopmental Follow-Up of Children-Administered Ketamine Prior to Cardiac Surgery in Infancy (SAFEKIDS study) (E0738801)

Responsible Division: Neurotoxicology
Objectives:

1) Assess the possibility of any long-term neuroprotective or neurotoxin effects in the previously studied population of patients who were enrolled as infants in the previous trial.
2) Administer neuropsychological tests after their 5th birthday to assess cognitive function, academia achievement, memory, operant behavioral endpoints an executive functioning.
3) Determine the behavioral outcomes of the cases and controls.

PI: Chen, James J., Ph.D.

Data-Mining Strategy to Identify Hepatotoxic Drugs and Sensitive Patients (E0740301)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Systems Biology
Collaborating FDA Center: CDER
Objective:
Build a prototype computerized visualization system to integrate FDA safety data-mining system for further analysis of the AERS data. The system will allow similarities and differences among drugs and among events to be explored for further investigation.

PI: Chen, Tao, Ph.D.

Development and Evaluation of Exposure Dosimetry Methods to Optimize the Standard *In Vitro* Mammalian Genotoxicity Assays for Assessing Engineered Nanomaterials (E0745701)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions/Office: Neurotoxicology, Systems Biology, Office of Scientific Coordination
Objectives:

1) Evaluate whether the *in vitro* mammalian genotoxicity assay is suitable for assessing the genotoxicity of nanomaterials.
2) Explore the possible mechanisms underlying genotoxicity of ENMs by conducting genomic analysis.
3) Identify potential improvements to the assay and general strategies for evaluating nanomaterials.
4) Examine whether the suitable methods and other experiences learned from the micronucleus assay are applicable to other genotoxicity tests, such as mouse lymphoma assay and *in vivo* micronucleus assay.

PI: Chen, Tao, Ph.D.

Development of a New Safety Evaluation Method Using MicroRNA (miRNA) Expression Analysis as a Biomarker for Detecting Carcinogens (E0728101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Systems Biology, Neurotoxicology
Objectives:

1) Determine miRNA expression

profiles of the tumor target-tissues of rats and mice treated with genotoxic carcinogens aristolochic acid (AA, riddelliine, and comfrey); and non-genotoxic carcinogens (propiconazole and triadimeforn), as well as non-carcinogen myclobutanil using microarray technologies.
2) Develop a polymerase chain reaction (PCR) array containing the primers that are specifically used to amplify carcinogensis-related miRNAs and use the PCR array to conduct time-course and dose-response studies for miRNA expression alterations in tissues of rats treated with carcinogens.
3) Define the miRNA biomarker genes that are associated with carcinogen exposure by prediction of their target genes and determination of their biological functions.

PI: Chen, Tao, Ph.D.

Do Engineered Silver Nanomaterials (Ag-ENMs) Varying by Size and Coatings Behave Differently Than Bulk Silver in Their Ability To Induce Genetic Damage? (E0750101)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Office of Scientific Coordination
Objectives:
1) Evaluate the Ames test and mouse lymphoma assay, in addition to the *in vitro* micronucleus assay.
2) Investigate Ag-ENMs of various sizes and compare to bulk silver results.

PI: Chen, Tao, Ph.D.

Evaluation of the Applicability of *In Vivo* Micronucleus Assays for Assessing Genotoxicity of Engineered Nanomaterials (E0731001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions/Office: Biochemical Toxicology, Microbiology, Neurotoxicology, Office of Scientific Coordination
Collaborating FDA Center: CFSAN
Objectives:
1) Assess the genotoxicity of four types of nanoscale materials, carbon nanotubes, nanoscale titanium dioxide, nanoscale gold, and nanoscale silver in three standard tests suggested by FDA; *Salmonella* Ames test, mouse lymphoma assay, and *in vivo* mouse micronucleus assay.
2) Evaluate the possible mechanisms of nanomaterial-induced genotoxicity using a transgenic mutation system, comet assay, and genomic analysis.

PI: Delclos, Kenneth B., Ph.D.

CD-1 Mouse Diet Pilot Study—Evaluation of Various Diets on Various Endpoints Criticial to Evaluation of Bisphenol A (BPA) and Other Endocrine Active Agents in Mice (E0218301)

Responsible Division: Biochemical Toxicology
Collaborating Division/Office: Personalized Nutrition and Medicine, Office of Scientific Coordination
Collaborating FDA Center: CFSAN
External Partner: National Toxicology Program
Objectives:

1) Determine a diet for assessing the effect of endocrine active compounds, including BPA, on reproductive and metabolic parameters in CD-1 mice.
2) Evaluate the role of soy protein, with or without the ethanol extractable components of soy that include the isoflavones, in the effects of soy-containing diets.
3) Conduct chemical and biological assays on the diets to determine the occurrence and variability of several component activities that could alter the *in vivo* response of the animal to such endocrine active compounds.

PI: Delclos, Kenneth B., Ph.D.

Dietary Modulation of the Renal Toxicity of p-Nonylphenol (NP) and Di(2-ethylhexyl)phthalate (DEHP) (E0714201)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Systems Biology, Personalized Nutrition and Medicine
Objectives:

1) Demonstrate that the incidence and/or severity of cystic kidney disease is decreased in rats fed soy-containing diets.
2) Evaluate the renal toxicity of dietary DEHP in developing rats maintained on a soy-free diet.
3) Evaluate potential early markers of renal cystogenesis in p-nonylphenol- and DEHP-treated rats and their modulation by soy-containing diets.
4) Evaluate the roles of modulation of antioxidant defenses and cyclooxygenase activities in the protective effect of soy against p-nonylphenol and, if demonstrated, DEHP-induced renal toxicity.
5) Assess the effect of diet on hepatic, testicular, and lung toxicity of DEHP.

PI: Delclos, Kenneth B., Ph.D.

Effects of Sedatives on the Metabolism of Di(2-ethylhexyl)phthalate (DEHP) Administered by Intravenous Injection and the Relationship of DEHP Metabolism to Biological Effects in Neonatal Rats (E0216201)

Responsible Division: Biochemical Toxicology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Centers: CBER, CDRH
Objectives:

1) Determine if sedatives potentially useful for intravenous-injection studies of DEHP in neonatal rhesus nonhuman-primates and/or in common use in neonatal intensive care units affect the metabolic profile of DEHP.
2) Examine DEHP metabolism in neonatal rodents dosed intravenously and orally and relate this metabolism to biological effects.

PI: Desai, Varsha G., Ph.D.

Development and Application of a Mitochondria-Specific Gene Array (Mitochip) for the Investigation of Clinical and Non-Clinical Predictive Biomarkers of Toxicity (E0739701)

Responsible Division: Systems Biology
Collaborating Divisions: Neurotoxicology, Personalized Nutrition and Medicine
Collaborating FDA Centers: CDER, CDRH
Objectives:

1) Develop MitoChip for various mammalian species, including rat, nonhuman primate, and human.

2) Conduct transcriptional profiling of mitochondria-related genes using mitochondria-specific gene arrays to investigate the mechanisms of drug toxicities and degenerative diseases associated with mitochondrial dysfunction.
3) Characterize species-specific transcriptional profiles to predict risk of drug toxicity or disease-onset in different mammalian species.

PI: Desai, Varsha G., Ph.D.

Development of a Translational Mouse Model of Drug-Induced Cardiac-Tissue Injury (P00744)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Personalized Nutrition and Medicine, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objectives:

1) Perform noninvasive measurements of heart rate, heart-rate variability, and electrocardiogram using ECGenie system.
2) Measure cardiac troponin T and creatine kinase MB in plasma/serum as indicators of DOX-induced cardiac-tissue damage.
3) Identify cardiac lesions by light microscopy and morphological changes in cardiac mitochondria by electron microscopy.
4) Measure levels of cardiolipin, amino acids, and Krebs cycle intermediates in serum using metabolomics.

PI: Ding, Wei, Ph.D.

Development of 3-D Human Skin Model for *In Vitro* Genotoxicity Testing of Chemical and Physical Agents (E0740001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Neurotoxicology
Collaborating FDA Center: CFSAN
Objective:

1) Evaluate the ability of 3-D human skin models to assess the genotoxicity of dermal exposure to FDA-regulated products, including nanomaterials.
2) Perform the Comet and MN assays with the 3-D human skin model to determine how each endpoint can be used to evaluate the genotoxicity of chemical and physical agents.

PI: Doerge, Daniel R., Ph.D.

Di(2-ethylexl)phthalate (DEHP) and Bisphenol A (BPA) Exposure in Pediatric Patients (E0742501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CDRH
Objectives:

1) Determine the pharmacokinetics of the production of urinary metabolites of DEHP and BPA after cardiopulmonary bypass (CPB) in children.
2) In a pilot study, quantify the exposure of children to DEHP and BPA while undergoing CPB compared to critically ill children without cardiac surgery and healthy controls.
3) Evaluate the ability of urinary biomarkers to detect acute kidney injury in patients following CPB.

PI: Doerge, Daniel R., Ph.D.

Human Biomonitoring for Bisphenol A (E0743101)

Responsible Division: Biochemical Toxicology

Objectives:

1) Develop and implement sensitive and selective analytical methodology to measure BPA from blood and urine samples from children and adults with known exposures.
2) Integrate human biomonitoring data with pharmacokinetic data from animals and humans to produce a PBPK model for BPA to empower FDA to reach science-based decisions about risks, particularly to children and fetuses, from medical devices, food contact materials, and other environmental exposures.

PI: Doerge, Daniel R., Ph.D.

Human Biomonitoring for Exposure to Bisphenol A (BPA) and Potential Replacement Products (NIEHS) (E0747101)

Responsible Division: Biochemical Toxicology

External Partner: National Toxicology Program

Objectives:

1) Provide human biomonitoring data for BPA and its structural analogs that are potential replacement products in adults exposed occupationally to thermal-paper cash register receipts. The routes of administration (dermal/oral) are likely key determinants of internal exposure to the active unconjugated form of BPA and/or possible replacements. These data will be used for physiologically based pharmacokinetic (PBPK) modeling along with existing pharmacokinetic data from experimental animals and humans.
2) Provide estimates of concentrations of active BPA aglycone in potential target tissues of developing fetuses and children for BPA and/or structural analogs from all possible exposures, particularly from food and medical devices, so FDA can make science-based decisions on risks from BPA and possible replacement products.

PI: Fang, Jia-long, Ph.D.

13-Week Dermal Toxicity of Triclosan in B6C3F1 Mice (E0217901)

Responsible Division: Biochemical Toxicology

Collaborating Division: Personalized Nutrition and Medicine

External Partner: National Toxicology Program

Objective:

1) Evaluate the toxicity of triclosan administered dermally to mice for 13 weeks.
2) Provide a scientific basis for dose selection for a subsequent chronic dermal-carcinogenicity study.

PI: Ferguson, Sherry A., Ph.D.

Investigation of Potential Functional Effects of Ventricular Enlargement in Sprague-Dawley Rats (E0744801)

Responsible Division: Neurotoxicology

Objective:

Assess four groups of Sprague-Dawley rats using two methods: 1) behavioral assessment using the Morris water maze and 2) measurement of ventricular size using MRI.

PI: Ferguson, Sherry A., Ph.D.

Long-Term Effects of Morphine Treatment in Preterm Infants Exposed to Repetitive Neonatal Pain (E0724301)

Responsible Division: Neurotoxicology
Objective:
Determine if neonatal intensive care morphine treatment in preterm infants is associated with long-term alterations in short-term memory and/or motivation at approximately 6 years of age.

PI: Fu, Peter P., Ph.D.

Use of Electron Spin Resonance (ESR) Spectroscopy to Characterize the Interactions Between Nanoscale Materials and Model Biological Systems (E0730601)

Responsible Division: Biochemical Toxicology
Collaborating FDA Product Center: CFSAN
Objectives:
1) Chemical Reactions
   a. Determine if nanomaterials can catalyze Fenton reaction to initiate hydroxyl radical formation in a nanoparticle size dependent manner.
   b. Determine if nanomaterials and/or their cations can be reduced by natural reducing agents, such as ascorbic acid and glutathione, leading to the formation of ROS.

2) Microsomal Metabolism Mediated Studies
   a. Determine if nanomaterials enhance or inhibit free radical formation, mediated by microsomal metabolism, in a nanoparticle size-dependent manner.
   b. Determine if nanomaterials and/or their cations can enhance dependent manner.

3) Cell-Culture Studies
   a. Determine the toxic effects, (including mitochondrial dehydrogenase activity, intracellular ROS concentration, and mitochondrial membrane potential) of nanomaterials of different particle size in cells, including A549 human-lung carcinoma cells and rabbit-brain rBCECs cells (a normal cell line to assay the toxic effect on the central nervous sytsem).
   b. Use ESR oximetry technique to determine the inhibition/induction of lipid peroxidation by nanomaterials of different particle size in A549 human-lung carcinoma cells and rabbit-brain rBCECs cells.

PI: Fuscoe, James, Ph.D.

Genetic and Epigenetic Mechanisms of Sex Differences in the Kidney of a Rat Model System: Developing Safety Biomarkers for FDA-Regulated Products (E0743901)

Responsible Division: Systems Biology
Collaborating FDA Office: FDA Office of Women's Health
Objectives:
1) Perform whole genome expression profiling on the 10 rat tissues of both sexes at 9 ages.
2) Perform miRNA profiling of selected tissues, including liver.
3) Perform DNA methylation profiling of selected tissues, including liver.

4) Use bioinformatics and statistical approaches to understand the genetic machinery operational at each developmental stage in each sex and relate the findings to potential susceptibility to adverse drug reactions and disease.
5) Use bioinformatics approaches to analyze the findings for potential age- and sex-related susceptibility in an animal model system to humans.

PI: Gough, Bobby J., Ph.D.

The Impact of a Glial Modulator (PPF) on Methamphetamine (METH)-Induced Dopamine Dynamics: A Microdialysis Study in Rats and Mice (E0743301)

Responsible Division: Neurotoxicology
Objectives:
1) Simultaneously measure DA and its metabolite levels in the caudate nucleus of rats and mice using dual online injection.
2) Determine effect PPF will have on METH-evoked DA levels.
3) Determine the protective nature of PPF against METH-induced neurotoxicity in both species. Results of pilot study will indicate possible future studies.
4) Strengthen FDA abilities in microdialysis and further validate the use of mice in neurochemical studies.

PI: Gregori, Luisa, Ph.D.

Rapid and Sensitive Detection of Creutzfeldt-Jakob Disease Agents in Tissue and Blood Donations (E0748901)

Responsible Division: Systems Biology
Collaborating FDA Center/Office: CBER, Office of Chief Scientist Challenge Grant
Objectives:
1) Develop a vCJD PrPTSE prototype test for blood donations.
2) Develop a prototype cornea donor test using brain-biopsy material.

PI: Gu, Qiang, Ph.D.

Identification of Protein Biomarkers for Neurotoxicity Assessments Using a High-Throughput Antibody Microarray Approach (E0747701)

Responsible Division: Neurotoxicology
Collaborating Division: Systems Biology
Objectives:
1) Examine proteomic changes at both the expression and phosphorylation levels using five established *in vivo* models of neurotoxicity.
2) Identify common changes in protein expression and phosphorylation status in these animal model systems.
3) Confirm the observed alterations in protein expression and phosphorylation status by means of other independent methods.
4) Apply the proteomic findings to a global ischemic animal model to further validate the utility of protein biomarkers for use in neurotoxicity assessments.

PI: Gu, Qiang, Ph.D.

Proteomic Assessment of the Cytotoxic Effects of Nanoparticles (NPs) on the Blood-Brain Barrier (BBB) (E0746001)

Responsible Division: Neurotoxicology
Collaborating Divisions/Office: Biochemical Toxicology, Neurotoxicology, Systems Biology, Office of Scientific Coordination
Objectives:
1) Examine silver, gold, cobalt, and chromium NPs emerging in biotech and medical applications.
2) Describe, using cutting-edge

proteomics approaches, alterations in expression and/or phosphorylation of proteins that are involved in apoptosis, inflammation, oxidative stress, and tumor-genesis signaling pathways in the cells that form BBB following NP exposure.

3) Correlate proteomic changes with conventional cytotoxicity and BBB permeability assays.

Additional NPs may be studied if early findings warrant, and if the project demonstrates utility in the approach, similar efforts will be applied in other studies to examine effects of NPs on other organs or cell types, for example the blood-placenta barrier.

PI: Hansen, Deborah K., Ph.D.

Development of the Mouse Embryonic Stem-Cell Test (E0741901)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Systems Biology
Collaborating FDA Center: CDER
Objective:
Gain hands-on experience with this test to better characterize it and indicate potential modifications to the assay.

PI: Hansen, Deborah K., Ph.D.

Dose-Finding Study for Reproductive and Developmental Toxicity Study of Oxybenzone (HMB) (E0217801)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Biochemical Toxicology
External Partner: National Toxicology Program
Objectives:

1) Determine doses of HMB to be used in future reproductive and developmental toxicity studies in rats which will be conducted according to guidelines set forth by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.
2) Determine plasma levels of HMB and its metabolites in the blood from treated pregnant dams.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Embryo/Fetal Development in Sprague-Dawley Rats (Segment II) (E0218701)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine, Biochemical Toxicology
External Partner: National Toxicology Program
Objectives:

1) Determine the potential developmental toxicity of oxybenzone.
2) Compare the results of a typical Segment I, II, III study with results from a modified one-generation study proposed by NTP.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Fertility and Early Embryonic Development in Sprague-Dawley Rats (Segment I) (E0218601)

Responsible Division: Systems Biology
Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine
External Partner: National Toxicology Program
Objectives:

1) Examine the reproductive toxicity of oxybenzone in male and female rats, focusing specifically on fertility and

early embryonic development to implantation.
2) Compare the results of a typical Segment I, II, III study design with results from a modified one-generation study proposed by the NTP.

PI: Hansen, Deborah K., Ph.D.

Effect of Oxybenzone on Pre- and Postnatal Development in Sprague-Dawley Rats (Segment III) (E0218801)

Responsible Division: Systems Biology
Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine
External Partner: National Toxicology Program
Objectives:
1) Study pre- and early postnatal development [ICH Guideline S5(R2) 4.1.2] to determine the potential toxicity of oxybenzone to male and female rats.
2) Compare the results of a typical Segment I, II, III study with results from a modified one-generation study proposed by the NTP.

PI: He, Zhen, Ph.D.

Brain Sexual Dimorphic Structures and Sex Hormone-Like Compounds (SHLC) (P00710)

Responsible Division: Neurotoxicology
Objective:
Establish a series of standardized procedures for evaluating SHLC-induced changes in brain morphology utilizing immmunohistochemical and other, more traditional techniques.

PI: Heflich, Robert H., Ph.D.

Development of a High-Throughput Assay for Measuring *In Vivo* Mutation in an Autosomal Gene (E0741301)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Objectives:
1) Develop a high-throughput *in vivo* mutation model that detects mutations induced by a range of mechanisms, including gene mutation, large deletions, and loss of heterozygosity.
2) Evaluate the basic properties and sensitivity of the model in experiments employing well-characterized mutagens.

PI: Heflich, Robert H., Ph.D.

Phosphatidylinositol Glycan Complementation Group A (Pig-a) Mutagenesis: An International Validation Study Comparing Pig-a Mutation in Rats with Other Biomarkers of Genetic Toxicity (E0741201)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Centers: CDER, CVM
Objectives:
1) Generate data using a standardized protocol that, in combination with results from other investigators, will be used to determine the sensitivity, specificity, and portability of the rat RBC/RET Pig-a gene mutation assay.
2) Determine how the RBC/RET Pig-a assay compares in terms of sensitivity and specificity with these other *in vivo* assays that have been used or considered for use as regulatory assays by performing the *in vivo* Comet, MN, and the Pig-a and

Hprt lymphocyte gene mutation assays in conjunction with the RBC/RET Pig-a assay.

PI: Hong, Huixiao, Ph.D.

Further Development and Refinement of the FDA Endocrine Disruptor (ED) Knowledge Base (EDKB) for Assessing Endocrine Disrupting Potential of Drugs and Food Additives (E0741501)

Responsible Division: Systems Biology
Collaborating Divisions/Office: Biochemical Toxicology, Office of Scientific Coordination
Objectives:

1) Improve EDKB by including the ED data that will be generated by NTP and NCTR as well as ER and AR data generated at the EPA, and data that have been published in the past 8 years.
2) Conduct metaanalyses of the large datasets accumulated in the EDKB to gain a better understanding of chemical structure requirement and mechanisms related to EDs.
3) Investigate various chemoinformatics/bioinformatics approaches to develop effective predictive models for assessing the potential of drugs and food additives.

PI: Howard, Paul C., Ph.D.

Analytical Assay for Photochemical Generation of Hydroxyl Radical (S00728)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Biochemical Toxicology
Objectives:

1) Provide support for analysis of the photoactivation of nanomaterials using the OH/coumarin-3-carboxylic acid assay.
2) Provide particle-size analysis for all materials being analyzed by OH method and other nanomaterials used in studies at NCTR and ARL/ORA.
3) Improve the assay using ultraviolet light diode laser as a replacement to the existing broad-band ultraviolet-light A source.

PI: Howard, Paul C., Ph.D.

Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Makeup (E0710501)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Objectives:

1) Determine the chemicals in tattoo pigments and their metabolism *in vitro*.
2) Develop methodology for tattooing SKH-1 hairless mice in a quantitative and reproducible manner.
3) Determine the extent of inflammation induced by the implanted pigment
4) Determine the time of recovery following tattooing.
5) Determine the acute toxicity of several tattoo inks and permanent makeup inks in SKH-1 hairless mice in the presence and absence of simulated solar light.
6) Determine if tattoo pigments are photocarcinogenic in the SKH-1 hairless mouse using simulated solar light.

PI: Inselman, Amy L., Ph.D.

Establishment of Embryonic Stem (ES) Cells as an *In Vitro* Model to Explore Developmental Toxicity (E0735401)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Maintain and passage several mouse ES and human induced pluripotent stem (iPS) cell lines in a pluripotent, undifferentiated state in the absence of feeder cells and serum.
2) Recapitulate early embryonic development by terminal differentiation of mouse ES and human iPS cells into a variety of cell types (i.e. osteoblasts).
3) Monitor this differentiation process by examining gene expression in undifferentiated ES and in cells that have undergone differentiation.
4) Provide proof-of-concept by using known teratogens and investigating gene changes in differentiated cells, such as acetazolamide treatment of differentiating osteoblasts.

PI: Kanungo, Jyotshnabala, Ph.D.

Effect of Pediatric Anesthetics on Zebrafish Embryos: Neurotoxicity vs. Gene Expression Changes and Neuronal Kinase (Cdk5) as a Mediator of Toxicity (E0736301)

Responsible Division: Neurotoxicology
Objective:

1) Determine if ketamine will have neurotoxic effects (on neurogenesis and axonogenesis) in zebrafish.
2) Determine if the window of such effects varies between early and late differentiating neurons (sensory and motor neurons, respectively).

PI: Kanungo, Jyotshnabala, Ph.D.

Methods Development for Toxicity Assays Using the Zebrafish Embryo as a Model System: Whole Animal High-Throughput Assays for Chemical Testing (E0735901)

Responsible Division: Neurotoxicology
Collaborating Office: Office of Scientific Coordination
Objective:
Establish a high-throughput assay system using zebrafish embryos to monitor both traditional morphological and behavioral endpoints of toxicity and the newer, more subtle organ-specific toxicities of FDA-relevant compounds.

PI: Lyn-Cook, Beverly A., Ph.D.

Genotyping Human Equilibrative Nucleoside Transporter 1 (hENT1) Polymorphisms in Normal and Pancreatic-Cancer Tissues: Assessing Gemcitabine and the Role of Indole-3-Carbinol in Chemosensitivity and Chemoresistance in Pancreatic Cancer (E0742701)

Responsible Office: Office of the Director/ADRA
Objectives:

1) Clarify the association of identified single nucleotide polymorphisms (SNPs) in hENT1 gene with hENT1 expression in human pancreatic-cancer tissue.
2) Identify SNPs in hENT1 gene in human pancreatic-cell lines and elucidate the association of SNPs in hENT1 gene with expression and activity of hENT1 in pancreatic-cancer cells.
3) Observe the effect of 13C and gemcitabine on hENT1 expression, activity and gemcitabine uptake in

pancreatic-cancer cells.

PI: Manjanatha, Mugimane, Ph.D.

Development of Methods for Evaluating DNA Damage Using Single-Cell Gel Electrophoresis (Comet Assay) in Rodents (E0729001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CFSAN
Objective:
Evaluate and establish methods and conditions that enhance the sensitivity and reproducibility of the *in vivo* alkaline-comet assay for use in preclinical-hazard identification and genotoxicity testing of food ingredients and chemicals for regulatory purposes.

PI: Manjanatha, Mugimane, Ph.D.

Validation of a Newly Developed Transgenic, Hairless, and Albino Mice (E0727701)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Office: Office of Scientific Coordination
Objectives:
1) Analyze tissue-specific mutant frequency and spectra using transgenic systems to evaluate a variety of hypotheses on the mechanisms or mode of action for cancer induction in rodents.
2) Facilitate improvements in human-risk characterization based on extrapolation from animal data.

PI: Mckinzie, Page B., Ph.D.

Development of Cancer-Relevant Biomarkers for Identification of Potential Carcinogens: Research To Understand the Normal Background Frequencies in Rats (E0733601)

Responsible Division: Genetic and Molecular Toxicology
Objective:
Understand the distribution and range of spontaneous oncogene-mutant frequencies in the major organs of rats and mice to provide important basic information for the validation of these oncogene-mutant frequencies as biomarkers of chemically induced carcinogenesis.

PI: Mendrick, Donna L., Ph.D.

Biomarkers of Liver Toxicity (E0732201)

Responsible Division: Systems Biology
Objectives:
1) Discover biomarkers of hepatotoxicity in preclinical studies that are more predictive of adverse effects in humans. These biomarkers may or may not be directly applicable to the clinic, but they will be predictive of human responses so that they can be used to extrapolate preclinical data to humans in safety assessments.
2) Qualify these biomarkers (e.g., via the FDA/EMEA qualification process) and potential translation for clinical use.

PI: Mendrick, Donna L., Ph.D.

Understanding and Predicting Immune-Mediated Idiosyncratic Drug Reactions (IDR): Molecular Modeling of Interactions Between Drugs, Polymorphic HLA Proteins, and T-Cell Receptors (E0739501)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objective:
Apply molecular modeling approaches to better understand the underlying mechanisms of existing drug-HLA combinations known to cause immune-mediated IDRs. Prediction models linking IDRs to specific interaction patterns between drugs and patient-specific polymorphic HLA and T-cell receptors (TCRs) may show enhanced predictability. The modeling approach, if proven to work for existing IDR cases, may be applicable to new drug-patient combinations.

PI: Moore, Martha M., Ph.D.

Development of a Method To Use *In Vivo* Mutagenicity Data to Address the Question as to Whether a Specific Chemical Induces Cancer via a Mutagenic or a Non-Mutagenic Mode-of-Action (MOA) (E0722901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Personalized Nutrition and Medicine
External Partner: Toxicology Excellence for Risk Assessment
Objectives:
1) Develop, evaluate, and disseminate a new NCTR method that utilizes *in vivo* mutagenicity and other key event data to address the question of whether a specific chemical induces cancer via a mutagenic or a non-mutagenic MOA.
2) Investigate design alternatives and various strategies for selecting doses and conducting the *in vivo* mutation studies required for this analysis.
3) Investigate modeling approaches for mutagenic and non-mutagenic MOAs to understand the relationship between the dose response for the induction of mutations and for tumors.
4) Evaluate several dose-response modeling methods (e.g., benchmark dose, categorical regression) to determine the appropriate model(s) for this analysis.
5) Develop the optimal experimental design and modeling methods to determine if a chemical is inducing cancer via a mutagenic MOA.
6) Share the utility of this new approach with the larger risk-assessment community.

PI: Moore, Martha M., Ph.D.

Evaluation of the Ability of Both the Agar and Microwell Versions of the Mouse Lymphoma Assay (MLA) to Optimally Detect the Mutagenic Potential and Potency of Complex Chemical Mixtures (E0728401)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Objective:
Develop science-based best-practice standard and tools to incorporate translational and applied toxicological advancements into the regulatory-science process to create a seamless bench-to-bedside continuum.

PI: Nakamura, Noriko, Ph.D.

Effect of Fetal Exposure to Oxybenzone on Reproductive Organs of Postnatal Day (PND)-21 Rats (E0745501)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Determine if fetal exposure to oxybenzone influences the male and female reproductive organs of Harlan Sprague Dawley rats on PND-21 by examining morphology of the reproductive organs and mRNA expression of genes related to the endocrine system.
2) Determine if fetal exposure to oxybenzone alters morphology of the testis and ovary of PND-21 rats.
3) Determine if fetal exposure to oxybenzone disrupts steroid biosynthesis of male reproductive organs of PND-21 rats.

PI: Ning, Baitang, Ph.D.

Using Existing Sequencing Data to Identify Genetic Variants Responsible for Corticosteroid-Induced Adrenal Suppression (P00754)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Evaluate genetic variants that are associated with corticosteroid-induced adrenal suppression.
2) Identify biomarkers that may lead to the safe use of corticosteroids in the treatment of children with asthma.
3) Build internal expertise to generate, evaluate, analyze, and interpret exome sequencing data.

PI: Parsons, Barbara L., Ph.D.

Evaluating the Utility of ACB-PCR in Dose-Response Assessment and Mode-of-Action Evaluation (E0726901)

Responsible Division: Genetic and Molecular Toxicology
Objectives:

1) Further develop, evaluate, and disseminate a new NCTR method, Allele-specific competitive blocker-PCR (ACB-PCR).
2) Determine whether ACB-PCR measurements of specific oncogenic base substitutions can be used to inform and improve the dose-response and mode-of-action assessments for cancer risk.

PI: Paule, Merle G., Ph.D.

Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery (E0703301)

Responsible Division: Neurotoxicology
Objective:
A battery of automated tests (games) will be given to measure aspects of learning, short-term memory and attention, motivation, time perception, and color and position discrimination.

PI: Paule, Merle G., Ph.D.

Implementation of a New Computer Configuration for Administration of NCTR's Operant Test Battery (P00746)

Responsible Division: Neurotoxicology
Objectives:

1) Develop a new USB-based system to replace and improve the droid system.
2) Compare the newly developed system with the older one to ensure continuity and compatibility.

PI: Paule, Merle G., Ph.D.

Long-Term Consequences of Neonatal Ketamine Anesthesia in Rhesus Monkeys: Extended Cognitive Assessments (E0736401)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDER
Objectives:

1) Continue monitoring the cognitive capabilities of rhesus nonhuman-primate subjects that were exposed to a single, 24-hour bout of ketamine-induced anesthesia during the first week of life. Data to date indicate that, compared to control animals, ketamine-exposed subjects exhibit significant deficits in several aspects of brain function, including learning, the ability to perform simple visual discriminations, motivation and speed of psychomotor processing. Continuing these observations will provide valuable information on the ultimate time course and severity of the observed deficits.
2) Extend the functional domains that are being assessed. Performance of a temporal discrimination task (timing task), a counting task and reversal learning tasks (cognitive flexibility) will be added to the current assessment battery.

PI: Petibone, Dayton, Ph.D.

Development of a Molecular Cytogenetics Laboratory in the Division of Genetic and Molecular Toxicology (E0734201)

Responsible Division: Genetic and Molecular Toxicology
Objectives:

1) Establish methods for the culture and harvest of peripheral blood lymphocytes, application of whole chromosome FISH probes to metaphase cells, and data collection and analysis.
2) Establish protocols for the culture and harvest of primary peripheral blood lymphocytes from nonhuman primates and peripheral blood lymphocytes, splenocytes, and bone marrow from rodents (mice & rats).

PI: Salminen, William F., Ph.D.

Evaluation of Growth and Pubertal Development in Male Rhesus Nonhuman-Primates (Macaca Mulatta) Chronically Exposed to Methylphenidate Hydrochloride (MPH) (E0728701)

Responsible Division: Systems Biology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology, Neurotoxicology, Personalized Nutrition and Medicine
Objectives:

1) Examine the genetic toxicity associated with chronic MPH treatment.
2) Continue dosing through the completion of puberty, to allow for evaluation of changes in pharmacokinetics and operant behavior testing.

PI: Shi, Qiang, Ph.D.

Preclinical Studies Investigating the Dose Range and Proof-of-Principle That Leflunomide-Induced Liver Injury (LILI) Is Enhanced by Cytochrome P450 Inhibition (E0744201)

Responsible Division: Systems Biology
Objectives:

1) Develop a rat model of LILI focusing on the involvement of hepatic cytochrome P450s.

2) Determine if a preclinical model of LILI can be established to help in the development of novel biomarker(s) to assess individual sucsceptibility to LILI in future studies.

PI: Shi, Qiang, Ph.D.

Using Cell-Free microRNA (miRNA) as Improved Clinical Biomarkers of Drug-Induced Liver Injury (DILI) (E0749701)

Responsible Division: Systems Biology
Collaborating FDA Center/Office: CDER, Office of Chief Scientist
Objective:

1) Measure the level of miRNAs in human serum, urine, and liver samples from patients experiencing DILI and normal healthy controls.
2) Compare the changes in miRNA levels between groups to identify specific miRNAs that may serve as new biomarkers of DILI.

PI: Tolleson, William H., Ph.D.

Photoinduction of Cutaneous Malignant Melanoma in TP-ras/ink4A (+/-) Transgenic Mice (E0708901)

Responsible Division: Biochemical Toxicology
Objectives:

1) Characterize photochemical DNA damage in the skin of TP-ras/ink-4a mice exposed to UVA+UVB radiation.
2) Determine whether cutaneous malignant melanoma can be induced in neonatal TP-ras (+) ink4a (+/-) transgenic mice using UVA+UVB radiation.
3) Identify photochemically induced mutations within the ink4a/p16/CDKN2A and p53 loci in tumor tissues.
4) Determine if UVA+UVB exposure at an early age creates a greater risk for developing cutaneous melanoma in TP-ras (+)ink4a(+/-) mice compared with chronic UVA+UVB exposure of older animals.

PI: Tong, Weida, Ph.D.

International Collaborations with the State Food and Drug Administration (SFDA) of the People's Republic of China on the Safety of Drugs and Medical Devices (S00742)

Responsible Division: Systems Biology
Objective:
Foster collaboration between FDA and SFDA with regard to monitoring and regulating the safety of drugs and medical devices through partnerships to better ensure the safety and quality of Drugs, Excipients, and Medical Devices.

PI: Ulmer, William C., Ph.D.

Support of CDER/Office of Counter Terrorism and Emergency Coordination "Animal Model" Project (S00753)

Responsible Office: Systems Biology
Collaborating FDA Center: CDER
Objective:
Provide support to CDER in the design and testing of a logical model and a physical-database schema derived from that model (Animal Model Database for Medical Countermeasures).

PI: Wang, Cheng, Ph.D.

Assessment of Gaseous Anesthetics in the Developing Nonhuman Primate (E0728501)

Responsible Division: Neurotoxicology
Collaborating Division/Office:
Biochemical Toxicology, Office of Scientific Coordination

Objectives:

1) Evaluate dose-response effects of gaseous anesthetics.
2) Determine if prolonged exposure to either nitrous oxide or isoflurane will result in an increase in neuronal-cell death.
3) Determine if combinations of nitrous oxide and isoflurane will prevent or enhance each other's effects on the developing nonhuman primate.
4) Determine if a relative high dose or prolonged exposure of the developing nonhuman primates to either nitrous oxide or isoflurane—or their combination—will induce long-term behavioral deficits, as well as long-lasting pathological changes.
5) Determine, using noninvasive imaging techniques [High resolution dedicated positron emission tomography (microPET) and magnetic resonance imaging (MRI)], if a high dose or prolonged exposure of the developing nonhuman primates to either nitrous oxide or isoflurane, or in combination, will induce long-lasting pathological changes. MRI will be used to verify pathological evidence and look for volume changes. MicroPET will be used to examine the sensitivity for tracing low picomolar concentrations of radiolabeled molecules, which is useful for studying dynamic imaging in animal models of human diseases.
6) Identify potential underlying mechanisms that could link alteration of mitochondrial function and elevation of reactive oxygen species to gaseous anesthetic-induced neuronal-cell death. L-carnitine will be used to attenuate neurological brain injury associated with mitochondria-related degenerative effects induced by gaseous anesthetics in the developing nonhuman primate.

PI: Wang, Cheng, Ph.D.

Assessment of Ketamine in the Developing Nonhuman Primate (E0718901)

Responsible Division: Neurotoxicology
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CDER
Objectives:

1) Determine, using neurohistochemical approaches, if, and at what developmental stages, ketamine exposure increases neuronal apoptosis/proliferation.
2) Determine, using neurohistochemical approaches, the dose-response for ketamine to produce apoptosis at the most sensitive developmental stage.
3) Determine the reversibility or permanence of the response using behavioral, imaging, and neurohistochemical approaches.
4) Determine, at the most sensitive stage and dose, genomic and proteomic responses to ketamine treatment.

PI: Wang, Cheng, Ph.D.

In Vitro Assay To Predict Developmental Neurotoxicity of Pediatric Anesthetics (E0740501)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDER
Objectives:

1) Examine additional anesthetics, including propofol (GABAA agonist),

baclofen (GABAB agonist), diazepam (GABAA agonist), pentobarbital (GABAA agonist & AMPA antagonist), etomidate (GABAA agonist), sevoflurane (NMDA antagonist & GABA agonist), fentanyl (opiate agonist), and anesthetic combinations commonly used in pediatric surgical procedures.

2) Determine the utility of *in vitro* culture systems to predict *in vivo* outcomes in subsequent studies.
3) Determine the dose and time-course over which the potential neurotoxic effects of anesthetics are expressed in the developing brain.
4) Determine effective ways to protect against anesthetic-induced developmental neurotoxicity that have potential clinical utility.
5) Identify mechanisms that link altered NMDA-receptor function and/or elevation of reactive oxygen species to anesthetic-induced neuroapoptosis.
6) Identify biomarkers, such as genomic-pathway signatures and determine their validity for predicting *in vitro* outcomes of pediatric anesthetic exposure.

PI: Wang, Cheng, Ph.D.

Methods Development for High-Resolution Dedicated Positron Emission Tomography (microPET) to Rodent Neuroplasticity and Toxicity During Development (E0726401)

Responsible Division: Neurotoxicology
Objectives:

1) Utilize microPET to screen and evaluate *in vitro* and *in vivo* measurements from a broad range of pathophysiological or pharmacological parameters using specific tracers in the developing rat. Three different age groups will be used.
2) Elucidate the relationship between apoptosis identifying ligands (specific tracers) and subsequent behavioral deficits.

PI: Wang, Cheng, Ph.D.

Neural Stem Cells, Developmental Biomarkers, and Biological Tools to Advance Mechanistic Understanding of Pediatric Anesthesia-Related Neurodegeneration (E0741701)

Responsible Division: Neurotoxicology
Objectives:

1) Examine the effects of general anesthetics, including ketamine (NMDA antagonist) propofol (GABAA agonist), baclofen (GABAB agonist), diazepam (GABAA agonist), sevoflurane (NMDA antagonist & GABA agonist), fentanyl (opiate agonist) and combinations commonly used in pediatric surgical procedures.
2) Determine the utility of *in vitro* human and/or rat stem cells to predict *in vivo* outcomes in subsequent studies.
3) Determine the dose and time-course over which the potential neurotoxic effects of anesthetics are expressed in these *in vitro* systems.
4) Determine effective ways to protect against anesthetic-induced developmental neurotoxicity that have potential clinical utility.
5) Identify mechanisms that link altered NMDA receptor function (e.g., changes in calcium flux) and/or increases in reactive oxygen species to anesthetic-induced

neuroapoptosis.

6) Identify biomarkers such as specific genes or biochemical pathways that might be useful for predicting *in vivo* outcomes of pediatric anesthetic exposure.

PI: Zhang, Xuan, Ph.D.

Assessment of the Pharmacokinetics, Pharmacodynamics, and Neurotoxic Effects of an Anesthetic in Juvenile Nonhuman Primates Undergoing Various Surgical Procedures (E0738101)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Objective:
Assess the pharmacokinetics, pharmacodynamics, and neurologic effects of ketamine anesthesia in neonatal primates undergoing surgery.

PI: Zhang, Yongbin, Ph.D.

Preliminary Studies on Detection of Nanoscale Gold, Zinc Oxide and Carbon Nanotube in Cultured Cells Using Confocal Raman Microscopy (P00752)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Systems Biology
Objectives:

1) Develop standard operating procedures for a) confocal Raman microscope imaging of nanoparticles or small molecules in cell cultures and b) darkfield microscopy with hyperspectral detection.
2) Determine the spectral characteristics of each nanomaterial in an aqueous system (water or agarose gels to mimic cellular conditions) using a) confocal Raman, b) darkfield microscopy with hyperspectral detection, and c) transmission electron microscopy (TEM) with EDS.
3) Determine the uptake of nano-Au, nano-ZnO and SWCNT in rat primary hepatocyte cells and macrophages using a) confocal Raman, b) darkfield microscopy with hyperspectral detection, and c) TEM with EDS.
4) Develop a manuscript comparing the specificity and sensitivity of the three techniques for the three nanomaterials, and share with NCTR and FDA research community.

PI: Beger, Richard, Ph.D.

Collaborative Interagency Development of *In Silico* Computational Toxicology Modeling To Predict Adverse Drug/Chemical Interactions with Cytochrome p450 Enzymes (E0734501)

Responsible Division: Systems Biology
Collaborating FDA Center: CDER
Objectives:

1) Develop *in silico* computational models to predict chemical interactions that can inhibit CYP3A4 and CYP2D6 enzymes.
2) Build structure-activity relationship and spectrometric data-activity-relationship models of chemical interactions that inhibit CYP3A4 and CYP2D6 enzymes.

PI: Beger, Richard, Ph.D.

Identification of New Mechanistic Biomarkers of Adverse Responses to Acetaminophen (APAP) (E0731301)

Responsible Division: Systems Biology
Objectives:

1) Identify specific adduct proteins in children/adolescents receiving therapeutic doses of APAP (P-adducts) and in children/adolescents that have received APAP overdoses (T-adducts).
2) Examine metabolomic markers in these patients to address the role of redox status and energy metabolism in the study population.
3) Establish 2nd-generation biomarkers of APAP toxicity using the data generated from this study, based on specific adduct proteins, which can be used in future risk assessment studies of children receiving APAP.

PI: Beger, Richard, Ph.D.

Natural History of Acute Kidney Injury (AKI) at Central Arkansas Veterans Healthcare System (E0726801)

Responsible Division: Systems Biology
External Partner: Central Arkansas Veterans Healthcare System
Objective:
Test clinical biomarkers of AKI identified from previous studies that precede the rise in serum creatinine and blood urea nitrogen (BUN) and can be measured in easily obtained urine samples from patients. The current clinic markers of AKI are serum creatinine and BUN and they arise only when significant renal damage has occurred. In acute situations, failure to have more sensitive biomarkers results in the inability to prioritize patients.

PI: Beland, Frederick A., Ph.D.

Carcinogenicity of Acrylamide and Its Metabolite (Glycidamide) in Rodents: Neonatal Mouse Bioassay (E0718501)

Responsible Division: Biochemical Toxicology
Collaborating Division: Genetic and Molecular Toxicology
Objective:
Compare the carcinogenicity of acrylamide and its metabolite glycidamide in B6C3F1 mice treated neonatally.

PI: Beland, Frederick A., Ph.D.

DNA Adducts of Tamoxifen (E0701101)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Microbiology, Neurotoxicology
Objective:
Characterize DNA adducts from suspected tamoxifen metabolites, and

develop methods for their detection and quantitation to determine if tamoxifen is acting through a genotoxic mechanism.

PI: Beland, Frederick A., Ph.D.

Liver Toxicity Biomarkers Study: Phase 1, Entacapone and Tolcapone (E0726601)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Personalized Nutrition and Medicine, Systems Biology
Collaborating FDA Center: CDER
Objective:
Establish liver-toxicity biomarkers and associated algorithms for use in preclinical drug development that will predict the probability of occurrence of hepatocellular injury at any subsequent phase of drug development or following approval of the drug for marketing. Emphasis will be placed upon drugs that do not demonstrate "classical" signs of liver toxicity during preclinical stages of drug development.

PI: Beland, Frederick A., Ph.D.

Two-Year Carcinogenicity Bioassay of Furan in F344 Rats (E0216801)

Responsible Division: Biochemical Toxicology
Collaborating Division: Personalized Nutrition and Medicine
External Partner: National Toxicology Program
Objective:
Determine the dose-response relationship for the carcinogenicity of furan in F344 rats.

PI: Buzatu, Dan A., Ph.D.

Analysis of Proton magnetic resonance spectroscopy (MRS) Data Using a Distributed Artificial Neural Network (E0719501)

Responsible Division: Systems Biology
Objective:
Evaluate whether a self-optimizing, parallel-distributed neural network can use the data from *in vivo* proton MRS exams to provide additional information about a brain lesion.

PI: Chang, Ching-Wei, Ph.D.

Integrated Analysis of Single Nucleotide Polymorphism (SNP) and Copy Number Variation in Genome Association of Breast Cancer (E0744401)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Office: FDA Office of Women's Health
Objectives:

1) Develop new statistical methods to integrate single locus changes (i.e., SNP) with DNA copy-number variation and assess association with breast-cancer status with these measures of genetic variability.
2) Identify genetic components of susceptibility to breast-cancer risk to allow early prevention and interventions to reduce suffering and loss of life due to breast cancer.

PI: Chelonis, John, Ph.D.

Complex Brain-Function Study in Children With and Without Major Depression (E0717701)

Responsible Division: Neurotoxicology
Objective:
Determine if children diagnosed with

major depression according to the Diagnostic and Statistical of Mental Disorders criteria perform differently than children without such a diagnosis on tests of motivation, simple visual discrimination, timing ability, memory, and learning.

PI: Chelonis, John, Ph.D.

Effects of Anxiety on Complex Brain Function in Children (E0721701)

Responsible Division: Neurotoxicology
Objective:
Determine if children with high levels of anxiety perform differently than children without anxiety on tests of motivation, simple visual discriminations, timing ability, memory, and learning.

PI: Chen, James J., Ph.D.

Benefit/Risk Classification Models for Regulatory Decision Making in Personalized Medicine (E0722001)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CDER
Objective:
Develop prediction models and computational methods for quantitative assessment of benefit/risk models for regulatory decisions in personalized medicine**.**

PI: Chen, James J., Ph.D.

Sex Differences in Molecular Biomarkers for Individualized Treatment of Non-Gender-Specific Disease: A Novel Classification Algorithm for the Development of Genomic Signatures from High-Dimensional Data (E0727901)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Find sex-specific high-dimensional biomarkers.
2) Develop classifiers for each sex using our CERP algorithm as well as several alternative algorithms.
3) Investigate the improvement in these high-dimensional biomarkers by using the variable importance derived from our classification algorithm to prioritize and combine features.
4) Find optimal cutoffs to select high-dimensional biomarkers and finalize the classification algorithm.
5) Assess the performance of sex-specific high-dimensional biomarkers from our classification algorithm by cross-validation in order to obtain a valid measure of prediction accuracy using publicly available high-dimensional non gender-specific data.
6) Develop a user-friendly web-accessed classification software tool.

PI: Desai, Varsha G., Ph.D.

Development of Predictive Mitochondrial Biomarkers for Drug-Induced Cardiotoxicity Using a System Biology Approach (E0733201)

Responsible Division: Systems Biology
Collaborating Division/Office: Biochemical Toxicology, Office of Scientific Coordination
Collaborating FDA Center: CDER
Objectives:

1) Measure heart rate and variability, using ECGenie.
2) Measure cardiac troponin T, creatine kinase MB, and cardiolipin levels in plasma as indicators of DOX-induced cardiac-tissue damage.

3) Identify morphological changes in cardiac mitochondria in left ventricular region by electron microscopy.
4) Use omics for heart-analyte profiling: transcriptional profiling of ~906 mitochondria-related genes using MitoChip; protein profiling by 2D-HPLC/MS/MS, and measurement of endogenous metabolites by NMR and MS.
5) Measure expression levels of 906 mitochondria-related genes in whole blood using MitoChip.
6) Measure levels of creatinine, creatine, lactate, Krebs cycle intermediates, small ketone bodies in plasma using metabolomics.
7) Integrate genomic, proteomic, and metabolomic endpoints in the heart tissue to define the molecular basis of DOX-induced cardiac toxicity and also correlate omics data to genomic findings obtained in whole blood.

PI: Desai, Varsha G., Ph.D.

Molecular Mechanisms Underlying Gender-Associated Differences in the Adverse Reactions to the Antiretroviral Agent, Zidovudine (AZT): Role of Mitochondrial Toxicity (E0725601)

Responsible Division: Systems Biology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Objective:
Elucidate molecular mechanisms of mitochondrial dysfunction that will address gender-based differences in adverse effects of antiretroviral drugs, such as AZT to provide FDA with data to develop treatment guidelines to reduce the frequency/severity of toxic effects in women (particularly pregnant women).

PI: Dobrovolsky, Vasily N., Ph.D.

Phosphatidylinositol Glycan - Complementation Group A (PIG-A) Mutagenesis: Development of Methods for the Identification and Molecular Characterization of Mutations in the PIG-A Gene in Human Lymphoblastoid Cells and C57Bl/6 Mice (E0720901)

Responsible Division: Genetic and Molecular Toxicology
Objectives:
1) Develop flow-cytometric methods for the detection of cells with mutations in the PIG-A gene using wild-type and mutant human lymphoblastoid cells, TK6, and WTK1, as a model.
2) Develop flow-cytometric methods for the detection of hematopoietic cells with mutations in the PIG-A gene in C57Bl/6 mice.

PI: Doerge, Daniel R., Ph.D.

Human Pharmacokinetics of Bisphenol A (BPA) (E0750001)

Responsible Division: Biochemical Toxicology
Objectives:
1) Measure deuterated BPA from blood and urine samples from adult humans after a single oral dose of 100 µg/kg bw in order to resolve uncertainty regarding human metabolism, pharmacokinetics (PK), and mass balance of BPA.
2) Integrate these new human PK and mass-balance data with the PK data from experimental animal models (e.g., mouse, rat, and monkey) along with human urinary biomonitoring data to refine a physiologically based pharmacokinetic (PBPK) model for BPA.

PI: Doerge, Daniel R., Ph.D.

Phytoestrogens and Aging: Dose, Timing, and Tissue (E0721001)

Responsible Division: Biochemical Toxicology
Objective:
Evaluate the potential benefits or detrimental effects of dietary phytoestrogens on breast cancer progression, adipose tissue, and the brain, using well-established laboratory animal models.

PI: Fang, Jia-long, Ph.D.

Vehicle Selection for Triclosan Dermal Toxicity Studies in B6C3F1 Mice (E0217501)

Responsible Division: Biochemical Toxicology
External Partner: National Toxicology Program
Objective:
Determine the appropriate vehicle for use in triclosan dermal-toxicity studies in B6C3F1 mice.

PI: Ferguson, Sherry A., Ph.D.

Methylphenidate (Ritalin) Exposure During Pregnancy: Assessment of Neurotoxicity in Offspring (E0731801)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology, Personalized Nutrition and Medicine, Systems Biology
Collaborating FDA Center: CDER
Objective:
Quantify the neurobehavioral toxicity associated with pre- and early postnatal treatment with methylphenidate in rats.

PI: Ferguson, Sherry A., Ph.D.

Neurobehavioral Effects of Bisphenol A Across Age and Sex (E0219201)

Responsible Division: Neurotoxicology
External Partner: National Toxicology Program
Objective:
Advance development of rapid detection technologies and testing platforms in the area of food safety, biosecurity, food biodefense, and bioterrorism.

PI: Fuscoe, James, Ph.D.

Assessment of the Global Gene-Expression Changes During the Life Cycle of Rats (E0712201)

Responsible Division: Systems Biology
Collaborating Divisions: Genetic and Molecular Toxicology, Personalized Nutrition and Medicine
Objectives:
1) Use the NCTR rat microarray chip to quantitate the relative expression of approximately 4000 genes in the liver of rats at different ages.
2) Verify the relative expression levels by quantitative PCR or Northern analysis.

PI: Fuscoe, James, Ph.D.

Characterization of Whole Genome Amplified (WGA) DNA for Use in Genotyping Assay Development (E0735201)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Collaborating FDA Center: CDRH
Objectives:
1) Determine WGA's impact on DNA copy number variation throughout the genome using the CFTR (cystic

fibrosis) gene as a model.
2) Determine the extent of mutations introduced into DNA by the WGA process. The CFTR gene will be used as a model system because it is a large gene (~200,000 bp) and is the target of genotyping assays in which it has been difficult to obtain rare-allele samples.

PI: Fuscoe, James, Ph.D.

Improved Prediction and Monitoring of Drug Safety Through Assessment and Simulation of Injury/Reserve/Repair Pathways (E0741101)

Responsible Division: Systems Biology
Collaborating FDA Center: CDER
Objectives:
1) Define the pathways associated with drug-induced injury, focusing on those reflecting the initial injury/repair process and the concept of "reserve.'"
2) Develop analytical tools and computer-based predictive models capable of identifying new classes of safety biomarkers that can serve as early signals of drug-induced injury and the amount of tissue-specific reserve available. These tools would be of particular utility in monitoring "hard to diagnose" toxicities that currently lack mechanistic understanding or a clear regulatory science path.

PI: Guo, Lei, Ph.D.

Study of Drug-Induced Liver Toxicity Using State-of-the-Art *In Vitro* Liver Models, Including Primary Rat and Mouse Hepatocytes and Stem Cells (E0732101)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Systems Biology
Objectives:
1) Obtain signature-gene and protein-expression patterns of each cell type for comparison to toxin-induced changes.
2) Determine the contribution of each cell type to overall liver toxicity from agent exposure once these isolated cell types are available reliably.
3) Provide training to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Hansen, Deborah K., Ph.D.

Delta Vitamin Obesity Prevention Summer Camp (E0733001)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions: Microbiology, Systems Biology
Objectives:
1) Analyze levels of 13 vitamins in 100 children in grades 4-6 to confirm food-frequency questionnaire data showing low intakes of certain nutrients and vitamins.
2) Provide fresh fruits, vegetables, and fortified snacks to supplement low-vitamin intake for a one-month period to improve serum concentration levels of vitamins.
3) Analyze ancestry through whole-genome scans and Candidate genes responsive to vitamin intake to associate individual responses with genetic polymorphisms.
4) Improve the nutrition and genetic education of the participants through lessons taught by local teachers with materials provided by NCTR, USDA, and local UAMS AHEC

diabetes educator.
5) Develop health-economic analyses of the intervention.
6) Begin developing a sustainable program for improving the foods of the children in the Marvell School District by analyzing economic impact of vitamin intervention.

PI: Hansen, Deborah K., Ph.D.

Developmental Toxicity of Environmental Contaminants in Folate-Deficient Mice (Preliminary) (P00696)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Objectives:
1) Collect preliminary data for an NIH grant submission.
2) Examine environmental conditions which may cause suboptimal delivery of folic acid to the fetus which can result in birth defects.

PI: Hart, Mark E., Ph.D.

Application of Co-Culture and Simulated-Vaginal Models to Elucidate the Inhibitory Properties of Naturally Occurring and Bioengineered Strains of *Lactobacillus* Toward Toxic-Shock Syndrome Toxin-1 (TSTT-1)Producing Strains of Staphylococcus aureus (E0728601)

Responsible Division: Microbiology
Collaborating FDA Center: CFSAN
Objectives:
1) Determine the inhibitory effects of a select group of *lactobacilli* with probiotic potential on a wide variety of *S. aureus* TSST-1 producing strains isolated from patients with Toxic Shock Syndrome using previously developed co-culture system and the vaginal tract (toroid) model with recently developed genital tract secretion medium.
2) Generate transcriptional and proteomic profiles of *Lactobacillus sp.* and *S. aureus* strains using previously developed co-culture system and identify gene systems and proteins critical for inhibition of *S. aureus* growth and/or TSST-1 production.
3) Isolate and clone the lysostaphin gene, an endopeptidase that specifically cleaves the cell wall cross-linking pentaglycine bridges of *S. aureus*, into a select group of *lactobacilli* and determine expression levels of lysostaphin as well as inhibitory capacity of engineered lactobacilli against *S. aureus* in the co-culture system and the vaginal tract (toroid) model.

PI: Hart, Mark E., Ph.D.

Co-Display of Hemagglutinin and CD154 on the Surface of Yeast Cells as a Vaccine Against Avian Influenza (E0733301)

Responsible Division: Microbiology
Objectives:
1) Generate HA surface presented yeast recombinant avian influenza vaccines.
2) Characterize humoral and cellular-mediated immune responses of yeast vaccines in mice.
3) Demonstrate protection of mice from lethal avian-influenza virus through yeast-based immunization.

PI: Hong, Huixiao, Ph.D.

Baseline Practices for Analyzing Genome-Wide Association Study (GWAS) Data (E0729701)

Responsible Division: Systems Biology
Collaborating FDA Center: CDER
Objective:
Conduct a comparative study of the latest methods for analyzing GWAS data with a focus on developing baseline practices.

PI: Imam, Syed Z., Ph.D.

Modulation of the Effects of Parkinson's Disease (PD) Medications by Nicotine (E0746601)

Responsible Division: Neurotoxicology
Objectives:
1) Evaluate the effect of nicotine treatment on the effect of PD medications in MPP+ treated DAN human dopaminergic cells and SHSY-5Y human neuroblastoma cell lines.
2) Evaluate the effect of nicotine administration on the effects of PD medications in an MPTP-treated C57BLJ6 mouse model of progressive PD.

PI: Khan, Ashraf A., Ph.D.

Screening of Dietary Supplements for *Bacillus* Contamination Using Chromogenic Agar: Identification of Enterotoxigenic *Bacillius Cereus* Group and Pre-Formed Emetic Toxin (E0748301)

Responsible Division: Microbiology
Collaborating FDA Center: CFSAN
Objective:
Improve the detection of enterotoxigenic *B. cereus* from dietary supplements and food products. Confirmation of the bacteria from incriminated dietary supplements and foods is complicated by the presence of background microorganisms. *B. cereus* is not competitive with other organisms; therefore, culture media with additional agents to suppress background microorganisms will be evaluated.

PI: Leakey, Julian E., Ph.D.

Subchronic Toxicity Studies of Chondroitin Sulfate and Glucosamine in Fischer 344 Rats and Diabetic Goto-Kakizaki Rats (E0215701)

Responsible Office: Office of Scientific Coordination
Collaborating Divisions/Office:
Biochemical Toxicology, Systems Biology, Office of the Center Director
Objectives:
1) Investigate the potential toxicity of chondroitin sulfate and glucosamine, administered by oral gavage in male rats.
2) Determine whether subchronic exposure of glucosamine or chondroitin sulfate potentiate the pathological effects of noninsulin-dependent diabetes in obese diabetic rats.

PI: Lyn-Cook, Beverly A., Ph.D.

Inactivation of UDP-Glucuronosyltransferases (UGT) in Human Breast Tissues: Assessing Cancer Risk, Tamoxifen Safety and Toxicity (E0734001)

Responsible Office: Office of the Center Director/ADRA
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CDER
Objectives:
1) Characterize UGT mRNA expression in normal and malignant human-

breast tissues isolated from the same donor and from different donors. This will be used to screen for interindividual differences in UGT expression and to determine the association of UGT expression and breast-cancer risk.

2) Identify polymorphisms in those UGT genes that show significant interindividual differences in UGT mRNA expression in all breast tissues. This will be used to determine if differences in UGT gene expression are affected by UGT genetic variations.
3) Determine the methylation profile of those UGTs identified in number two above and correlate it to UGT expression.
4) Determine the effects of polymorphisms in UGT genes on glucuronidation of E2, 4-OH-E1, and 4-hydroxy-Tamoxifen using glucuronidation-activity assay and MTT-cytotoxicity assays.

PI: Lyn-Cook, Beverly A., Ph.D.

Sex and Ethnic Differences in Expression of Toll-Like Receptors (TLR-3, TLR-7, and TRL-9) in Systemic Lupus Erythematous: New Targets for Emerging Therapeutics (E0744601)

Responsible Office: Office of the Center Director/ADRA

Collaborative FDA Office: FDA Office of Women's Health

Objectives:

1) Determine the expression levels of Toll-like receptors 3,7, 9, and miRNA-146a in controls and lupus patients grouped according to sex and ethnicity.
2) Correlate expression of TRLs 3,7, 9 to type 1 interferon (IFN) levels in lupus and control patients.
3) Determine the polymorphisms profile of TRLs 3,7, and 9 in lupus and control patients and correlate to expression levels.
4) Correlate expression levels of TRLs 3,7, and 9 with expression of miRNA-146a.
5) Determine if IFN regulation may be through epigenetic regulation.

PI: Lyn-Cook, Beverly A., Ph.D.

Sex Differences in Chemotherapeutic Toxicity: Profiling of Transporter Genes in Human Liver (E0725401)

Responsible Office: Office of the Center Director/ADRA

Collaborating Divisions: Personalized Nutrition and Medicine, Systems Biology

Objectives:

1) Identify sex differences in the gene expression of drug transporters known to be involved in the transport of chemotherapeutic drugs and with hepatic expression in human-liver tissues. This is a prerequisite to elucidating the mechanisms of interindividual variability in hepatic drug transport systems.
2) Evaluate sex-related hepatic drug-transport functions, including both of the basolateral-transport systems that are responsible for translocating drugs across the sinusoidal membrane and the active canalicular transport systems that are responsible for the biliary excretion of drugs using sandwich-cultured human hepatocytes.
3) Characterize the relationships

between transporter gene expression and uptake or excretion of chemotherapeutic drugs defined with the sandwich model and transporter-transfected cell lines.

4) Evaluate the effects of sex hormones on hepatic-transporter gene expression in human cancer-cell lines and sandwich-cultured hepatocytes.
5) Identify and validate novel transporter-drug correlations using a chemogenomic approach followed by cytotoxicity and drug-uptake studies in cell lines overexpressing specific transporter genes.
6) Develop an *in silico* pharmacokinetic (PK)-modeling program based on the data from sandwich-cultured hepatocytes to predict potential *in vivo* drug PKs and toxicity in men and women.
7) Develop guidelines and recommendations for clinical-trial design and analysis of sex differences in new drug applications.

PI: Lyn-Cook, Beverly A., Ph.D.

Sex Differences in Systemic Lupus Erythematosus (SLE): Effects of a Single Nucleotide Polymorphism (SNP) in the Prolactin Gene on Individual Response to Prasterone Therapy (E0727401)

Responsible Office: Office of the Center Director/ADRA

Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine, Neurotoxicology

Objective:

Elucidate whether the PRL -1149G SNP increases SLE susceptibility by modulating signal-transduction pathways in a manner reversible by prasterone.

PI: Lyn-Cook, Beverly A., Ph.D.

The Role of Sex in Expression of DNA Cytosine 5-Metyltransferases, Histone Deacetylases, Acetylases, Metyltransferases, and Demethylases Among Patients with Systemic Lupus Erythematosus (SLE): Elucidating Potential New Drug Targets (E0738601)

Responsible Office: Office of the Center Director/ADRA

Objective:

Elucidate whether there is a sex and/or ethnic bias in expression levels of epigenetic markers in SLE patients.

PI: Mei, Nan, Ph.D.

Development of a New T-cell Receptor (TCR) Gene Rat Model for Safety Screening of Pharmaceuticals and Other Chemicals for Potential Mutagenicity (E0719601)

Responsible Division: Genetic and Molecular Toxicology

Objectives:

1) Develop an *in vivo* model using the TCR genes of the Fisher 344 rat for the rapid, cost effective, and predictable identification of pharmaceuticals and other chemicals that can induce mutations.
2) Use model mutagens, N-ethylnitrosourea (ENU) and cyclosphosphamide (CP) to investigate the potential utility of the TCR gene-mutation assay using isolated spleen lymphocytes derived from treated Fisher 344 rats.
3) Compare the mutant frequencies in the TCR genes and the Hprt gene in spleen lymphocytes of rats after mutagen exposure to validate the TCR assay.

PI: Moore, Martha M., Ph.D.

Further Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay (MLA) and the Role of the Assay in Mechanistically Based Risk Assessment (E0711701)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Objectives:

1) Determine if the L5178Y/TK+/- Mouse Lymphoma Assay adequately detects both aneuploidy and mitotic recombination.
2) Determine if the L5178Y mouse lymphoma cells have active recombinase functions which lead to a large proportion of mutants that result from recombinase-mediated rearrangements.
3) Determine the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.

PI: Morris, Suzanne M., Ph.D.

Animal Models of Pregnancy To Address Medical Countermeasures for Influenza and Chemical, Biological, Radiological and Nuclear Threats in the "At Risk " Population of Pregnant Women — Phase I (E0746201)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Biochemical Toxicology, Systems Biology
Collaborating FDA Center: CDER
Objectives:

1) Conduct literature search and analysis regarding animal models of pregnancy.
2) Organize/sponsor public workshop.

PI: Morris, Suzanne M., Ph.D.

Effect of p53 Genotype on Gene-Expression Profiles in Mice Exposed to the Model Mutagen, N-ethyl-N'-nitrosourea (ENU) (E0712901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Biochemical Toxicology, Microbiology, Personalized Nutrition and Medicine, Systems Biology
Objectives:

1) Determine the effect of mutation in the p53 tumor-suppressor gene on gene-expression profiles in young and aged mice, and the effect of those exposed to the model mutagen, N-ethyl-N-nitrosourea.

PI: Myers, Meagan B., Ph.D.

Determining Oncomutation Profile of Triple Negative Breast Cancer: Information to Direct Developement of Personalized Therapies (E0743801)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CDRH
Collaborating FDA Office: FDA Office of Women's Health
External Partner: University of Arkansas for Medical Sciences
Objectives:

1) Establish which molecules should be targeted to treat the largest percentages of breast cancers.
2) Identify which mutational biomarkers should be used as diagnostics in personalized approaches to breast- cancer treatment.
3) Determine what sensitivity is needed in the measurement of those mutational biomarkers.

PI: Ning, Baitang, Ph.D.

Mechanisms of Gender Differences in Aspirin Effects: Metabolizing Enzymes and Therapeutic Targets (E0727101)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Objectives:

1) Profile gender differences in the mRNA expression and protein production of drug-metabolizing enzymes known to be involved in aspirin metabolisms, using human liver samples from 50 males and 50 females.
2) Characterize molecular mechanisms of sex hormones (estrogens, progestogens, and androgens) in regulation of the expression of aspirin-metabolizing genes in human ER-positive hepatic-cell line HepG2-ER(+) using biochemical procedures, including DNA-protein binding assay and reporter construct assay.
3) Measure sex-hormone modulation of aspirin effect on platelet aggregation and its related biomarkers (COX-1, COX-2, PGE2, TXA2, and LTB4) using human platelet precursor cells.
4) Identify sex-hormone modulation of aspirin actions in human endothelial and epithelial cell lines, by measuring prostacyclin dynamics (PGE2, TXA2 and LTB4) and aspirin-targeting enzymes (COXs, NOSs, and LOX) expression.
5) Evaluate sex-hormone modulation of response to aspirin in apoliprotein E-deficient mice.

PI: Ning, Baitang, Ph.D.

Micronutrient Involvement in Differentiation of Multipotent Mesenchymal Stem Cells into Adipocytes through MicroRNA Regulation (P00720)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions: Genetic and Molecular Toxicology, Sytems Biology
Objectives:

1) Identify microRNA biomarkers of adipogenesis process.
2) Investigate the effect of micronutrients on microRNA expression during the differentiation process of mesenchymal stem cells into adipocytes.

PI: Ning, Baitang, Ph.D.

Whole-Genome Sequencing to Identify Genetic Susceptibilities to Carbamazepine-Induced Adverse Reactions (E0745301)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Systems Biology
Objectives:

1) Identify and compare genetic variants in 30 SJS/TEN patients and 10 Amish individuals with public data from the 1000 Genome Project.
2) Identify genetic variants associated with phenotypes of interest.
3) Evaluate the molecular mechanisms accounting for inter-individual variations responding to carbamazepine.
4) Model patient-specific drug-HLA interactions to predict the outcome.
5) Assess technical performance and bioinformatics solutions of NGS on whole-genome sequencing.

PI: Parsons, Barbara L., Ph.D.

Analysis of p53 Codon 270 CGT to TGT Mutation in Simulated Solar Light (SSL)-Induced Skin Tumors and Exposed Mouse Skin (E0715201)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Divisions: Biochemical Toxicology**,** Personalized Nutrition and Medicine
Objectives:

1) Develop the ACB-PCR detection of mouse p53 codon 270 CGT->TGT mutation.
2) Measure the frequency of detection and levels of this mutation in mouse-skin tumors.
3) Measure the frequency of this mutation in skin tissue from tumor-bearing animals.
4) Measure the frequency of this mutation in skin exposed to decreasing levels of SSL.

PI: Parsons, Barbara L., Ph.D.

Cancer Mutations as Biomarkers of Cancer Risk: Human Studies with Implications for Personalized Medicine (E0726501)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Develop the information necessary for the rational use of oncogene mutations as quantitative biomarkers of cancer risk; specifically Allele-specific competitive blocker PCR (ACP-PCR) will be used to determine normal and pathological levels of relevant oncogene mutations in multiple human tissues and tumors.
2) Compare the information derived from human tissues with data generated in a parallel rodent protocol as an approach for incorporating carcinogenesis-relevant data into the rodent to human extrapolation necessary in cancer-risk assessment.
3) Validate a streamlined ACP-PCR methodology and to develop the methodology necessary to measure oncogene MF in cell-free DNA isolated from plasma.
4) Convey to the regulatory risk-assessment community through a series of publications, the regulatory significance of the data regarding tumor-associated mutations which have and will be generated.

PI: Paule, Merle G., Ph.D.

Novel Studies on Sites-of-Action and Mechanisms in Chronic Balance Dysfunction (E0722301)

Responsible Division: Neurotoxicology
Objective:
Develop and implement a comprehensive assessment of all levels of the neuraxis in an effort to determine central nervous system deficits due to balance disorder and vertigo and develop and assess strategies to restore those deficits.

PI: Petibone, Dayton, Ph.D.

Differential Transcriptomic Characterization of TK6 and WTK1 Human Lymphoblast Cells by Next-Generation RNA Sequencing (E0744001)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology

Objectives:

1) Develop next-generation pyrosequencing capabilities for RNA-sequence analysis and genomic sequencing in order to characterize the TK6 and WTK1 transcriptosome.
2) Apply technology to the determination of the baseline whole-genome gene expression levels in each cell line.
3) Measure transcriptosome response in TK6 and WTK1 after exposure to classical positive control agents which may include the direct-acting alkylating agent, ENU, as well as ionizing radiation. Other positive control agents may be selected during the conduct of the protocol.

PI: Pogribny, Igor P., Ph.D.

Development of a Targeted MicroRNA-Based Epigenetic Approach for Breast Cancer Treatment (E0746101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Personalized Nutrition and Medicine, Systems Biology
Collaborating FDA Office: FDA Office of Women's Health
Objectives:

1) Show that assessing expression of miRNAs that target DNA methyltransferases and other components of DNA methylation machinery will improve diagnosis of breast cancer.
2) Demonstrate that correcting miRNA function will restore the expression of epigenetically silenced genes in breast cancer, inhibit breast cancer progression, and improve the therapeutic efficacy of existing conventional chemotherapeutic drugs.

PI: Pogribny, Igor P., Ph.D.

Global and Locus-Specific DNA Hypomethylation: A Common Mechanism Involved in Genotoxic and Non-Genotoxic Rat Hepatocarcinogenesis (E0718101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Objectives:

1) Determine if the temporal alterations in genomic methylation profile in preneoplastic liver tissue observed in the folate/methyl-deficient model of rat endogenous hepatocarcinogenesis also occur in other carcinogenesis model.
2) Identify genes that are consistently up-regulated or down-regulated in target tissue during the promotion stage of carcinogenesis.
3) Evaluate whether or not the global and locus-specific DNA hypomethylation, along with aberrant expression of related genes and changes in chromatin conformation is specific only to target tissues and may be used for early detection of chemicals with carcinogenic potential.

PI: Pogribny, Igor P., Ph.D.

Relationship Between Liver Epigenetic Phenotype and Susceptibility to Nonalcoholic Steatohepatitis (NASH)-Induced Hepatocarcinogenesis in Mice (E0735301)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Objectives:

1) Determine the role of epigenetic dysregulation in the etiology and

pathogenesis of dietary NASH-induced hepatocarcinogenesis in mice.
2) Determine whether or not interstrain-specific susceptibility of mice to NASH-induced hepatocarcinogenesis is associated with differences in individual hepatic epigenetic phenotypes.
3) Determine the role of epigenetic dysregulation in the etiology and pathogenesis of NASH-induced hepatocarcinogenesis in mice induced by tamoxifen administration.
4) Determine if aberrant epigenetic markers can be used as targets for prevention of NASH-induced hepatocarcinogenesis in mice.

PI: Sarkar, Sumit, Ph.D.

Evaluation and Characterization of Blood-Brain Barrier (BBB) Pathology in MPTP-Probenecid-Induced Parkinsons Disease (PD)-Like Conditions in a Mice Model and its Potential Amelioration by Endoplasmic Reticulum Stress Reducers (Molecular Chaperones) and Other Putative Anti-PD Therapeutics (E0745101)

Responsible Division: Neurotoxicology
Objectives:
1) Determine the role of key NVUs in the expression of PD-like pathology.
2) Determine the ability of ER stress-reducers to alter the expression of PD-like pathology.
3) Determine the ability of antioxidant peptide SS31, N-acetyl cysteine, acetyl-l-carnitine, the orexin A receptor inhibitor SB 334 867 A, and metal chelators such as M30, clioquinol, and VK-28 to provide neuroprotection against PD-like pathology.
4) Evaluate changes in cerebral hemodynamics, BBB permeability and neurochemicals associated with the development of PD-like pathology.
5) For those compounds shown to be efficacious in ameliorating PD pathology, behavioral assessments will be conducted to quantify symptom improvement.

PI: Schmued, Laurence C., Ph.D.

Development of Novel Histochemical Markers of Brain-Vascular Elements and Their Application for Localizing Neurotoxicant-Induced Pathologies (E0731201)

Responsible Division: Neurotoxicology
Collaborating Office: Office of the Center Director
Objectives:
1) Develop and characterize novel markers for brain-vascular elements and investigate the effects of three different classes of neurotoxicants viz., kainic acid, an NMDA agonist, 3-nitropropionic acid (3-NPA), an inhibitor of metabolic respiration and metamphetamine, a dopamine agonist on each of the above-mentioned vascular elements such as perivascular pericytes, vascular lumen and perivascular sheath.
2) Characerize the response of certain vascular elements to neurotoxic insults.
3) Provide fluorescent and bright field labeling at the vascular lumen.

PI: Schmued, Laurence C., Ph.D.

Histochemical Test Battery for Evaluating the Efficacy and Toxicity of Putative Alzheimer's Disease (AD) Therapeutics of FDA Relevance (E0727301)

Responsible Division: Neurotoxicology
Objectives:

1) Test the hypothesis that AD, which is characterized by the deposition of insoluble amyloid plaques in the brain, is the result of a cascade of pathological processes and that pharmacological intervention at various points could attenuate the resulting pathology. To do this, the protocol will look at 13 different potential AD therapeutics, each with known but different mechanisms of action.
2) Obtain efficacy and toxicity data on 12 of the more promising AD therapeutic agents, including many presently undergoing Phase 2 or 3 FDA clinical trials.

PI: Shi, Leming, Ph.D.

Phase II of the MicroArray Quality Control Project (MAQC-II) Toward Personalized Medicine (S00705)

Responsible Division: Systems Biology
Collaborating Division: Biochemical Toxicology
Objectives:

1) Assess the reliability of microarray-based predictive models for clinical and preclinical applications.
2) Share consensus recommendations with the microarray community.
3) Facilitate the appropriate application of microarray data in the discovery, development, and review of FDA-regulated products.

PI: Shi, Leming, Ph.D.

SEQC (MACQ-III) —The Sequencing Quality Control Project (E0731901)

Responsible Division: Systems Biology
Collaborating Divisions: Genetic and Molecular Toxicology, Personalized Nutrition and Medicine
Collaborating FDA Centers: CFSAN, CDER, CDRH
Objectives:

1) Assess different next-generation sequencing (NGS) technologies and various bioinformatics strategies for handling and analyzing the massive sequence datasets by using the reference RNA samples previously established by the MAQC project.
2) Profile, using NGS technologies, RNA samples isolated from cells with or without treatment by nanoparticles and known toxicants to further evaluate their performance in assessing the safety and toxicity of FDA-regulated products.

PI: Shi, Qiang, Ph.D.

Identifying Drugs That Cause Female-Biased Hepatotoxicity by Analyzing FDA Drug-Approval Packages/Labels and FDA-Maintained Databases and Conducting Comparative Studies in Primary Hepatocytes of Rats, Mice, and Humans (E0750201)

Responsible Division: Systems Biology
Collaborating FDA Office: FDA Office of Women's Health
Objectives:

1) Identify specific drugs that cause drug-induced liver injury (DILI) more often in women than in men.
2) Establish a hepatocyte culture model to study a drug's potential to induce sex-biased DILI.

PI: Sonko, Bakary, Ph.D.

Evaluation of Glycolysis and TCA Fluxes in MPTP-Treated C57BL Mouse Model of Parkinson's Disease (PD) (E0732601)

Responsible Division: Systems Biology
Objectives:

1) Determine fluxes of 13C-glucose in the glycolysis pathway and through the TCA cycle in MPTP C57BL mouse model of PD.
2) Use the data to estimate the contributions of glycolysis and TCA-cycle pathways to energy metabolism in the model.
3) Identify potential energy metabolic biomarkers of PD in this setting.

PI: Stingley, Robin L., Ph.D.

Biomarkers for Early Detection of Kidney Damage in Hypertension (E0747301)

Responsible Office: Office of Scientific Coordination
Objectives:

1) Determine if a suite of biomarkers of acute kidney injury can be used to track the progression of nephropathy in the spontaneously hypertensive rat (SHR) and obese SHR (SHROB) animal models.
2) Characterize the relationship between biomarker expression in the kidney and expression of markers of oxidative and nitrosative stress in the kidney.

PI: Sun, Jinchun, Ph.D.

Preclinical Metabolomic Investigation of Drug Pharmacokinetics in Multiple Drug Toxicity Studies (E0732401)

Responsible Division: Systems Biology
Objectives:

1) Apply metabolomic methods to investigate a drug-metabolite profile in urine samples from preclinical studies using LC/MS and NMR with the combination of principal component analysis (PCA) and heterocorrelation analyses of NMR and mass spectrometer (MS) data.
2) Determine the excretion kinetics of the drug-N-acetyl-cysteine conjugates and S-adenosylmethionine, which is the primary source of the sulfur atom in the biosynthesis of glutathione using LC/MS/MS technique.
3) Investigate mercapturic acids profile using a highly sensitive and selective constant neural-loss technique developed on a triple quadrupole MS.

PI: Tong, Weida, Ph.D.

Development and Refinement of the FDA Genomic Tool, ArrayTrack™ for Advancing Pharmacogenomics and Personalized Medicine Supporting FDA's Critical Path Initiative (S00671)

Responsible Division: Systems Biology
Collaborating Division: Personalized Nutrition and Medicine
Objectives:

1) Analyze data from CDER drug review offices using ArrayTrack™™ and return results to CDER collaborators.
2) Develop the functionality in ArrayTrack™ to review non-microarray PGx data, supporting the Critical Path Initiative.
3) Develop new modules in ArrayTrack™ to review proteomic, metabolomic, and genome-wide association studies data.
4) Develop modules to allow electronic data submission in the VGDS/VXDS program.

PI: Tong, Weida, Ph.D.

Development of an FDA Resource and Knowledge Base for Sex Differences in Drug-Induced Liver Injury (DILI) (E0733801)

Responsible Division: Systems Biology
Collaborating FDA Center: CDER
Objectives:

1) Develop a standard data-curation model for the sex-biased DILI in ArrayTrack™ to manage the collected data. The model will be developed in accordance with the data standard developed in FDA for electronic data submission.
2) Augment further, the collection of the genomic data from public resources and through collaborations.
3) Conduct the meta-analysis, text mining, and network analysis to develop a relationship between drugs, molecular signatures, liver-specific biomarkers, genes/proteins functions, pathways, and sex-biased liver toxicity.

PI: Tong, Weida, Ph.D.

Development of Liver Toxicity Knowledge Base (LTKB) to Empower the FDA Review Process (E0721501)

Responsible Division: Systems Biology
Collaborating Division/Office: Biochemical Toxicology, Office of the Director
Objectives:

1) Liver Ontology (LO)—Develop an LO that characterizes liver pathology and toxicity and will be used as guidance for data collection/curation, classification and analysis described below.
2) Gene Expression Data—Collect existing gene-expression data. Other types of data such as data from proteomics, metabonomics, and genotyping studies (including GWAS) will be considered as the project progresses.
3) Text Mining—Conduct text mining on >13 million abstracts in PubMed and other public resources with an emphasis on liver-related data. The association between the liver-specific entities (i.e., genes/proteins, pathways, drugs, tissues and toxicity) will be established.
4) Known Data—Assemble knowledge available in public domains on liver toxicity, including genes/proteins, pathways/networks, and chemicals/drugs in such a way that it can be integrated with information in LTKB and effectively mined.
5) Experiment—Conduct gene-expression studies on well-understood and characterized hepatic and non-hepatic compounds. The dataset will be used to validate the LTKB. Data from 50 compounds will be generated in the first year and 150 additional compounds will be collected in the following two years, assuming the first set of data supports the LTKB approach.
6) LTKB—Analyze (combined and correlated) data collected in 1-5 above to establish liver toxicity-related regulatory networks and genes/proteins-pathways-chemicals-disease associations.

PI: Tong, Weida, Ph.D.

MicroArray Quality Control (MAQC) Project Database (S00691)

Responsible Division: Systems Biology
Objectives:
1) Update MAQC database when new data become available.
2) Maintain and regularly back up database at NCTR.

PI: Varma, Vijayalakshmi, Ph.D.

An Omics Approach To Investigate the Metabolic and Endocrine Effects of Fructose on Adipocytes Compared to Clucose (E0740401)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Systems Biology
Collaborating FDA Center: CFSAN
External Partner: University of Arkansas for Medical Sciences
Objective:
Identify cellular mechanisms involved in fructose-induced metabolic and endocrine regulation of human adipocytes in culture using omic technologies.

PI: Varma, Vijayalakshmi, Ph.D.

Epigenetics, DNA Methylation, and Obesity (E0733101)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Division: Systems Biology
Objective:
Evaluate the effect of differences in DNA methylation and agouti signaling protein in the offspring of Avy/a dams x a/a sires as a result of nutrient x gene interactions. These preliminary data will be used to select the appropriate diets for further studies on obesity and type 2 diabetes.

PI: Varma, Vijayalakshmi, Ph.D.

Yellow Agouti Mouse Breeding Colony (E0728801)

Responsible Division: Personalized Nutrition and Medicine
Objective:
Analyze minimum nutritional requirements for maintaining health and preventing disease.

PI: Wagner, Robert D., Ph.D.

Effects of Phytoestrogens on Gene-Expression Responses of Vaginal Epithelial Cells After Contact With *Candida Albicans* (E0740601)

Responsible Division: Microbiology
Collaborating FDA Center: CFSAN
Objective:
Examine whether phytoestrogens affect the ability of vaginal epithelial cells to mobilize host defenses against *C. Albicans* through changes in receptor-mediated signal transduction.

PI: Wagner, Robert D., Ph.D.

Maintenance of Germ-Free BALB/c Mice in Isolators for Use in Future Protocols (E0736701)

Collaborating Division: Microbiology
Objective:
Maintain a small colony of germ-free BALB/c mice in gnotobiotic isolators between approved research protocols.

PI: Wilkes, Jon G., Ph.D.

Quantum Mechanical and NMR-Spectral Approaches for the Rapid Prediction of Estrogen Activity of FDA-Regulated Chemicals (E0740701)

Responsible Division: Systems Biology
Collaborating FDA Center: CFSAN
Objective:
Provide a tool for new drug reviewers or

to retrospectively evaluate grandfathered drugs with respect to estrogeneic activity using available artificial neural networks trained on objective data and used to evaluate a broad structural range. Models could be added to the NCTR Endocrine Disruptor Knowledgebase or provided directly to CDER and other FDA Product Centers.

PI: Yu, Li-Rong, Ph.D.

Methods for Support of a Functional Proteomics Facility at NCTR (E0713501)

Responsible Division: Systems Biology

Objectives:

1) Establish and standardize for routine-use, procedures for whole-cell and subcellular organellar isolation for a variety of tissues.
2) Develop and standardize specific and sensitive markers of cell type and organellar purity and yield.
3) Identify, adapt, develop, and standardize appropriate 2-D protein separation techniques.
4) Integrate results to provide "front-end" components of a functional proteomics facility.

Strengthen Surveillance and Risk Analysis

PI: Azevedo, Marli, Ph.D.

Development of an Infectivity Assay to Detect Human Norovirus from Contaminated Food (E0745801)

Responsible Division: Microbiology
Objectives:

1) Develop alternative assays to detect infectious norovirus from contaminated food.
2) Gain insights on norovirus *in vitro* replication.

PI: Beland, Frederick A., Ph.D.

Effect of Urinary pH on Nephrotoxicity of a Combined Exposure to Melamine & Cyanuric Acid (E0731501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objective:
Determine effect of urinary pH on the renal toxicities from combined exposure of melamine and cyanuric acid.

PI: Beland, Frederick A., Ph.D.

Genotoxicity and Carcinogenicity of Acrylamide and its Metabolite (Glycidamide) in Rodents: Rangefinding/ 2-Yr Carcinogenicity Studies (E0215001)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Personalized Nutrition and Medicine
External Partnership: National Toxicology Program
Objective:
Compare the carcinogenicity of acrylamide and its metabolite–glycidamide–in B6C3F1 mice and Fischer 344 rats treated chronically for 2 years.

PI: Binienda, Zbigniew K., Ph.D.

Assessment of Iron-Oxide Nanoparticle (NP)-Induced Neurotoxicity in Cell Cultures and Whole-Animal Models (E0739401)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CFSAN
Objectives:

1) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce specific changes in the mitochondrial function, cell death, and generation of ROS in different regions of rat and mice brain using *in vivo* microdialysis.
2) Determine if acute or chronic exposure to iron-oxide NPs produce significant changes in neurotransmitter concentrations in different regions of mice/rat brains using microdialysis.
3) Determine if acute or chronic exposure of different sizes of iron-oxide NPs produce alterations in the brain-free fatty acid levels.
4) Determine if acute or chronic exposure to different sizes of iron-oxide NPs produce changes in lipid peroxidation and/or in antioxidant enzyme activity (catalase, superoxide dismutase, glutathione peroxidase) and glutathione levels in mice & rat brains.
5) Determine if acute or chronic exposure of different sizes of iron-oxide nanoparticles produce selective pattern of deposition and damage produces in different regions of rat and mice brain using *in vivo* MRI.

PI: Boudreau, Mary D., Ph.D.

13-Week Study To Determine the Pathogenesis of the Whole-Leaf Extract of the *Aloe Vera* in the Cecum and Large Intestine of the F-344 Rat (E0218201)

Responsible Division: Biochemical Toxicology
External Partner: National Toxicology Program
Objectives:

1) Determine the fraction(s) of the *Aloe vera* whole-leaf extract responsible for the non-neoplastic lesions observed in the 13-week study and the carcinogenic effects observed in the two-year bioassay.
2) Evaluate whether other extracts of the *Aloe vera* plant, including *Aloe* gel of the inner parenchyma leaf tissue and *Aloe* decolorized whole-leaf extract, an extract of the whole leaf of *Aloe vera* but treated subsequently with activated carbon, exert similar effects in the rat large intestine.
3) Examine whether Senna, a dietary supplement with components similar to those found in *Aloe vera*, exerts comparable effects in the rat intestine when administered in the drinking water.

PI: Boudreau, Mary D., Ph.D.

Bioassays in the Fischer 344 Rat- and the B6C3F1 Mouse-Administered *Aloe vera* Plant Constituents in the Drinking Water (E0214201)

Responsible Division: Biochemical Toxicology
Collaborating Divisions: Genetic and Molecular Toxicology, Microbiology, Personalized Nutrition and Medicine
Objective:
Conduct bioassays in rats and mice using standardized preparations of *Aloe vera* to explore the limits of safety for the *Aloe vera* leaf constituents present in commercial products. The use of *Aloe vera* is not limited to over-the-counter dermal therapeutics and cosmetics. It is also taken for internal consumption and used as a prophylaxis and treatment for a variety of unrelated systemic conditions.

PI: Buzatu, Dan A., Ph.D.

FERN Level-One Validation Study of a Mobile, Field-Rugged Rapid Detection and Enumeration System for *Salmonella* in Foods (E0731601)

Responsible Division: Systems Biology
Collaborating Divisions: Microbiology, Personalized Nutrition and Medicine
Objectives:

1) Conduct a FERN (Food Emergency Response Network) level-one validation for LITMUS Rapid Identification of Bacterial Pathogens (RAPID-B) screening of viable pathogens in food. This technology originated in the NCTR Division of Systems Toxicology.
2) Qualify the system for rapid screening of bacteria-contaminated food in FDA Office of Regulatory Affairs laboratories and mobile field response units, especially the FDA FERN network. The first validation exercise will be conducted by Arkansas Regional Laboratory. Pending successful completion of the level 1 validation, the study will be expanded to CFSAN, OARSA.

PI: Camacho, Luisa M., Ph.D.

Assessment of Molecular Changes in Male and Female Sprague-Dawley Rats Orally Exposed to Bisphenol A (BPA) from Gestation-Day 6 Through Postnatal-Day 90 (E0218401)

Responsible Division: Biochemical Toxicology
External Partner: National Toxicology Program
Objective:
Determine BPA-induced molecular changes (gene expression, protein levels, and epigenetic modifications) in tissues collected from Sprague-Dawley rats orally exposed to BPA from gestation-day 6 through postnatal-day 90.

PI: Cerniglia, Carl E., Ph.D.

Evaluate the Impact of Deepwater Horizon Oil-Contaminated Gulf Seafood Residues in Edible Tissues on the Human Intestinal Microbiota of the Consumer (E0742801)

Responsible Division: Microbiology
Collaborating FDA Center: CFSAN
Objectives:
1) Determine whether PAH residues in edible tissues of Deepwater Horizon oil-contaminated seafood adversely affect the human intestinal microbiota.
2) Determine if the human intestinal microbiota metabolize polycyclic-aromatic hydrocarbons (PAHs) that are toxic components of Deepwater Horizon oil.
3) Identify, characterize, and determine the toxicity of PAH metabolites generated from degradation by human intestinal microbiota.

PI: Cerniglia, Carl E., Ph.D.

Does the Human Intestinal Microbiota have the Enzymatic Capacity to Metabolize Melamine to Cyanuric Acid (P00730)

Responsible Division: Microbiology
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CVM
Objectives:
1) Determine if melamine impacts the population dynamics of the human intestinal microbiota.
2) Determine if the human intestinal microbiota metabolizes melamine to cyanuric acid.

PI: Chen, Huizhong, Ph.D.

Evaluation of Product and Physiologic Variables Influencing Smokeless Tobacco Toxicity (E0747201)

Responsible Division: Microbiology
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology
Collaboring FDA Center: CTP
Objectives:
1) Determine the effect of smokeless tobacco on oral bacterial ecology.
2) Assay and compare the toxicity and genotoxicity of smokeless tobacco before and after metabolism by oral bacteria.
3) Demonstrate the metabolism of tobacco-specific compounds, N-nitrosamines by oral bacteria.
4) Evaluate the relationship of smokeless tobacco on the antibiotics resistance of oral bacteria.

PI: Chen, James, Ph.D.

Integrated Genomics Knowledge Base for Rapid Threat Assessment of Enteric Pathogens: *Salmonella* (E0733701)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions/Office: Microbiology, Systems Biology, Office of the Center Director/OR
Objective:
Develop an integrated phenotypic and genotypic knowledge base for detection and characterization of *Salmonella*, and potentially other foodborne pathogens.

PI: Delclos, Kenneth B., Ph.D.

Evaluation of Molecular, Morphological, and Functional Endpoints in NCTR Sprague-Dawley Rats Treated with Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley Rats from Gestational Day 6 until Birth and Directly to Pups from Postnatal Day (PND)-1; Continuous and Stop Dose (PND-21) Exposures (E0219101)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objectives:

1) Evaluate a range of molecular, morphological, and functional endpoints in rats dosed orally with a wide range of BPA doses in a chronic toxicology study.
2) Determine if any effects observed are predictive of long-term toxic effects evaluated in the companion chronic toxicology study or reveal potential effects undetected by standard toxicological evaluations.

PI: Delclos, Kenneth B., Ph.D.

Evaluation of the Toxicity of Bisphenol A (BPA) in Male and Female Sprague-Dawley Rats Exposed Orally from Gestation Day 6 through Postnatal Day-90—Subchronic II (E0217601)

Responsible Division: Biochemical Toxicology
Collaborating Division/Office: Personalized Nutrition and Medicine, Office of Scientific Coordination
Collaborating FDA Center: CFSAN
External Partner: National Toxicology Program
Objectives:

1) Characterize the dose-response for orally administered BPA in the NCTR Sprague-Dawley rat to address the question of adverse effects in rodents near levels of exposure potentially attainable in humans. The potent orally active estrogen, ethinyl estradiol will be included as a reference to demonstrate the estrogen responsiveness of the animal model under these exposure conditions.
2) Evaluate endpoints related to the reported effects of BPA on other organ systems, including the development of obesity and cardiovascular disease.

PI: Delclos, Kenneth B., Ph.D.

Two-Year Chronic Toxicology Study of Bisphenol A (BPA) Administered by Gavage to Sprague-Dawley rats from Gestational Day-6 until Birth and Directly to Pups from Post-Natal Day (PND)-1, Continuous and Stop Dose (PND-21) Exposures (E0219001)

Responsible Division: Biochemical Toxicology

Collaborating Office: Office of Scientific Coordination
External Partner: National Toxicology Program
Objective:
Characterize the long-term toxicity of orally administered BPA, including developmental exposure, in the NCTR Sprague-Dawley rat over a broad dose range. In addition, animals generated in this study will be assigned to separate protocols for assessment of a range of molecular, morphological, and functional endpoints to determine if these endpoints are predictive of long-term toxic effects or reveal potential effects undetected by standard toxicological evaluations.

PI: Doerge, Daniel R., Ph.D.

Determination of Carcinogenic Mechanisms for Furan in Fischer 344 Rats (E0216401)

Responsible Division: Biochemical Toxicology
Collaborating Division: Genetic and Molecular Toxicology
Objectives:

1) Develop and validate LC-ES/MS/MS assays to quantify the major furan-derived DNA adducts in liver, the major furan-derived hemoglobin adduct(s), and the major furan-derived urinary glutathione-derived metabolite.
2) Determine dose-response relationships for liver furan-derived DNA and hemoglobin adduct formation, and repair/turnover and the major furan-derived urinary glutathione-derived metabolite in male and female Fischer 344 rats following single- and multiple-dose exposures of rodents to furan.
3) Determine concentration of furan in irradiated NIH-31 diet using headspace-GC/MS.
4) Determine toxicokinetics of furan in male and female Fischer 344 rats following exposure by single gavage administration using headspace-GC/MS.
5) Combine all data from single- and repeated-dose toxicokinetics of furan in rat blood and liver with the corresponding levels of liver DNA and hemoglobin adducts, and urinary mercapturates to construct a PBPK model to determine carcinogenic risks from human exposure to furan through the diet.
6) Determine mutagenicity of furan in liver *in vivo* using male Big Blue rats.
7) Determine the dose-response relationships for furan-mediated hepatotoxicity and cell proliferation in liver of male and female Fischer 344 rats.
8) Determine effects of furan on methylation status in rat liver and kidney DNA, and histones as epigenetic changes related to carcinogenic process.

PI: Doerge, Daniel R., Ph.D.

Effect of Soy-Containing Diets on Ammonium Perchlorate-Induced Thyroid Toxicity in Sprague-Dawley Rats: II (E0742201)

Responsible Division: Biochemical Toxicology
Objective:
Determine the effect of dietary whole soy and purified genistein, the principal soy isoflavone, on the dose-response characteristics for perchlorate-induced thyroid toxicity in male Sprague-Dawley rats. A critical processing error in the

original project makes it necessary to repeat the study. The results from the previous study, while incomplete and therefore insufficient to inform regulatory policy, are very provocative and largely substantiate the original hypothesis that soy diets can adversely affect thyroid function in the presence of additional risk factors.

PI: Doerge, Daniel R., Ph.D.

Human Studies of Isoflavone Safety and Efficacy (S00607)

Responsible Division: Biochemical Toxicology
External Parners: University of Miami, Wayne State University
Objective:
Conduct bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.

PI: Doerge, Daniel R., Ph.D.

The Role of Perinatal Development on Toxicokinetics of Bisphenol A (BPA) (E0216701)

Responsible Division: Biochemical Toxicology
Collaborating Divisions:
Neurotoxicology, Personalized Nutrition and Medicine
Collaborating FDA Center: CDRH
Objectives:

1) Determine BPA pharmacokinetics at low dose (100 ug/kg bw single dose; 100 ug/kg bw/d repeated).
2) Measure free and conjugated forms of BPA separately.
3) Use deuterium-labeled BPA to avoid issues of background contamination.
4) Use LC/MS/MS for sensitivity and selectivity of measurement.
5) Determine complete rat dataset for blood, tissue, and excreta across stages of development (pregnant females, fetuses, neonates).
6) Determine BPA pharmacokinetics from oral and intravenous administration in pregnant, lactating, and non-pregnant female rats, and in neonatal rats.
7) Determine plasma and urinary pharmacokinetic data in neonatal and adult nonhuman-primates.
8) Use the new pharmacokinetic data in conjunction with literature data from experimental animals and humans to build a physiologically based pharmacokinetic model for BPA with the ultimate goal of predicting target-tissue concentrations of active BPA in humans, including fetuses and children, from food and medical-device exposures.

PI: Ferguson, Sherry A., Ph.D.

Training for BPA Studies (P00706)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine
Objective:
Develop the skills and techniques, such as complex behavioral assessments and quantitative volumetric analysis of sexually dimorphic brain regions to conduct subsequent studies of developmental treatment with BPA.

PI: Fisher, Jeffrey W., Ph.D.

Biological Based Dose-Response (BBDR) Modeling for the Thyroid Axis in the Fetus and Neonate (E0743601)

Responsible Division: Biochemical Toxicology
Objectives:

1) Create BBDR models for the HPT axis

in the developing rat and human as a function of iodide status.
2) Interface the BBDR-HPT models with PBPK or TK models for thyroid active chemicals to predicted conditions (iodide status and chemical exposure) for which brain thyroid-hormone homeostasis cannot be maintained in the fetus and neonate.
3) Evaluate the possible influence of population exposures to thyroid active chemicals on fetal and neonatal-thyroid status as a function of iodide intake using the models.

PI: Fisher, Jeffrey W., Ph.D.

PBPK Models for BPA (E0742601)

Responsible Division: Biochemical Toxicology

Objectives:

1) Create physiologically based pharmacokinetic (PBPK) models for BPA in mouse, rat, and rhesus nonhuman-primate of adult, neonatal, pregnant (mother and fetus), and lactating (mother and neonate) laboratory animals. These models will be used to calculate internal measures of dose for both active and inactive forms of BPA.
2) Create human PBPK models for BPA (adult, child, pregnant mother and fetus, and lactating mom and infant) using data from the nonhuman-primate, mouse, and rat, and limited human information from literature. The human suite of models will be used to extrapolate the internal toxic doses of BPA in laboratory animals to humans. The PBPK models will also be used to extrapolate dosimetry from regions of observation to low levels of exposure to BPA for which no experimental data exist.
3) Interpret biomonitoring data for BPA in urine and blood.

PI: Foley, Steven L., Ph.D.

Characterization of Plasmid-Associated Antimicrobial Resistance in *Salmonella Enterica* Serovars Associated with Poultry and Human Infections (E0733501)

Responsible Division: Microbiology
Collaborating Office: Office of the Center Director/OR
Collaborating FDA Center: CVM
Objectives:

1) Identify and understand the genetic mechanisms associated with plasmids that facilitate the spread and persistence of virulence and multidrug resistance in *Salmonella* from poultry- and egg-associated serovars.
2) Sequence the plasmids from multidrug-resistant *S. enterica* serovar *Enteritidis, Heidelberg*, and *Typhimurium* strains to identify genes likely associated with virulence and antimicrobial resistance.
3) Determine the relative selective potential of antimicrobial agents to trigger the dissemination of antimicrobial resistance and virulence factors to susceptible *Salmonella.*
4) Determine the contribution of plasmids transferred via conjugation to virulence in *Salmonella* strains.

PI: Fu, Peter P., Ph.D.

Mechanism of Tumorigenic Pyrrolizidine Alkaloids and Development of LC-ES-MS/MS Methodology for Detection and Quantification of Pyrrolizidine Alkaloids (E0728901)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Objectives:

1) Validate the proposed mechanism by which pyrrolizidine alkaloids induce tumors in rodents.
2) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived DNA adducts in rodents.
3) Develop an LC/ES/MS/MS method for detection and quantification of genotoxic pyrrolizidine alkaloids in herbal plants and herbal dietary supplements.
4) Develop an LC/ES/MS/MS method for detection and quantification of DHP-derived hemoglobin adducts in rodents.

PI: Fu, Peter P., Ph.D.

Method Development for Study of Antioxidant Properties in Dietary Supplement (E0730501)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objectives:

1) Microsomal Metabolism Mediated Studies
    a. Determine whether or not the studied herbal dietary supplements can enhance or inhibit free-radical formation, mediated by microsomal metabolism, in a dose-dependent manner.
    b. Determine whether or not the studied herbal dietary supplements can enhance or inhibit microsomal metabolism mediated lipid peroxidation in a dose-dependent manner.
2) Cell-Culture Studies
    a. Determine the toxic effects, including mitochondrial dehydrogenase activity, intracellular ROS (reactive oxygen species) concentration, and mitochondrial membrane potential, of the studied herbal dietary supplements in cells, including A549 human-lung carcinoma cells and rabbit-brain rBCECs cells (a normal cell line to assay the toxic effect on the central nervous system).
    b. Determine, using the ESR oximetry technique, the inhibition/induction of lipid peroxidation by the studied herbal dietary supplements in A549 human-lung carcinoma cells and rabbit-brain rBCECs cells.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Assessment of the Nephrotoxic Effect of a Combined Exposure to Melamine and Cyanuric Acid (E0216901)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Collaborating FDA Centers: CFSAN, CDRH, CVM

External Partner: National Toxicology Program

Objectives:

1) Conduct a pharmacokinetic study on F344/N rats on the absorption and disposition of melamine and cyanuric acid when administered individually by gavage, simultaneously as a separate base and acid, and simultaneously as a pre-formed salt (melamine cyanurate).
2) Determine the NOAEL of a combined exposure to melamine and cyanuric acid in F344/N rats for 28 and 90 days.
3) Investigate the occurrence of early metabonomic and proteomic biomarkers of melamine and cyanuric acid-induced nephrotoxicity obtainable by noninvasive methods.
4) Investigate the pharmacokinetics and determine the NOAEL of a combined exposure to melamine and cyanuric acid in a mini-pig model deemed to be representative of the human-kidney anatomy and physiology.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Assessment of Nephrotoxicity from a 90-day Combined Exposure to Melamine and Cyanuric Acid in F344 Rats (E0218101)

Responsible Division: Biochemical Toxicology

External Partner: National Toxicology Program

Objective:

Determine the dose-response relationship for the nephrotoxicity of a 90-day combined exposure to melamine and cyanuric acid in F344 rats.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Assessment of Nephrotoxicity from an Exposure to Melamine, Cyanuric Acid, and its Combination in Newborn F344 Rats from Postnatal Day-1 to Weaning (E0218901)

Responsible Office: Biochemical Toxicology

Collaborating FDA Centers: CFSAN, CVM

External Partner: National Toxicology Program

Objectives:

1) Determine if a combined exposure to melamine and cyanuric acid in newborn F344 rats is more nephrotoxic than exposure to the individual compounds.
2) Establish the dose-response curve for the combined exposure.
3) Investigate the longer-term effects during a recovery period.

PI: Gamboa Da Costa, Goncalo, Ph.D.

Assessment of the Nephrotoxicity of a Seven-Day Combined-Exposure to Melamine and Cyanuric Acid (E0731701)

Responsible Office: Biochemical Toxicology

Collaborating FDA Centers: CFSAN,CVM

Objective:

Investigate the nephrotoxic effect of a seven-day co-exposure to melamine and cyanuric acid in Fischer 344 rats.

PI: Guo, Lei, Ph.D.

Develop Methods for the Evaluation of Smokeless Tobacco-Associated Carcinogenesis (E0748801)

Responsible Division: Biochemical Toxicology

Collaborating Division: Microbiology

Collaborating FDA Center: CTP

Objectives:

1) Evaluate and compare the carcinogenic activity of smokeless tobacco products.
2) Investigate animal models for comparing and evaluating carcinogenic activities (especially oral-cavity tumor induction) of smokeless tobacco products.
3) Test the hypothesis that tobacco-specific N-nitrosamines (TSNA) are major contributors to carcinogenic activity of smokeless tobacco.
    a. Determine and quantify the major carcinogenic alkaloid-derived tobacco-specific N-nitrosamines (TSNA) (NNK and NNN) in each product.
    b. Detect and quantify NNK-and NNN-derived DNA adducts in various samples, such as liver, pancreas, blood, and oral tissue, collected from animals administered NNK, NNN, or smokeless tobacco.
4) Determine gene expression and DNA methylation profiles at whole genome level and for specific pathways, such as DNA damage/repair, for biomarker discovery and mechanism elucidation.
5) Determine effect of smokeless tobacco products or TSNA on oral microbiota of the animals.

PI: Guo, Lei, Ph.D.

Toxicokinetic Studies of Berberine in SKH-1 Hairless Mice and *In Vitro* Phototoxicity Testing for Berberine and Goldenseal: Phase I for Phototoxicity and Photocarcinogenicity Studies of Goldenseal and Berberine (E0217701)

Responsible Division: Biochemical Toxicology

Objectives:

1) Study the photocarcinogenic effects of orally administered berberine and goldenseal in SKH-1 mice exposed to UVA light.
2) Determine tissue distribution of berberine in SKH-1 mice following oral administration.

PI: Hanig, Joseph P., Ph.D.

Development of MRI Imaging and Informatics Techniques for Tissue Sampling to Guide and Confirm Classical Neuropathology (E0741801)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CDER
Objective:
Use MRI and informatic analysis of MRI files to screen brain samples for neuro-irregularities (presumed toxicities) that would inform and direct the plane and loci of slices or sections taken for confirmatory classical neuropathology.

PI: Hansen, Deborah K., Ph.D.

Developmental Toxicity of Bitter Orange in Rats (E0214701)

Responsible Division: Personalized Nutrition and Medicine
Collaborating Divisions: Biochemical Toxicology, Genetic and Molecular Toxicology, Systems Biology
Collaborating FDA Center: CFSAN
Objective:
Determine potential developmental toxicity of synthetic synephrine and citrus aurantium extract in rats.

PI: Hansen, Deborah K., Ph.D.

Physiological Effects of Bitter Orange in Rats (E0214901)

Responsible Division: Personalized Nutrition and Medicine

Collaborating Divisions: Genetic and Molecular Toxicology, Systems Biology
Collaborating FDA Center: CFSAN
Objective:
Determine potential physiological effects of synthetic synephrine, as well as an extract from the botanical citrus aurantium alone, and in combination with caffeine in rats.

PI: Harris, Stephen C., Ph.D.

Scientific Enclave, Knowledge Base, and Topic Data Mining for Tobacco Products (E0749001)

Responsible Division: Systems Biology
Collaborating FDA Center: CTP
Objectives:
1) Develop a scientific enclave housing software platforms for collaborative information and data exchange between FDA and collaborators both within and outside of HHS.
2) Develop a Tobacco Constituents Knowledge Base (TCKB).
3) Develop a scientific data-mining algorithm for textual data on tobacco products.

PI: Heflich, Robert H., Ph.D.

Evaluating the Toxicity of Tobacco Products Using *In Vitro* 3-D Tissue Models (E0746801)

Responsible Division: Genetic and Molecular Toxicology
Collaborating Division: Systems Biology
Collaborating FDA Center: CTP
Objectives:
1) Identify the available engineered-tissue models that may be applicable to evaluating the toxicity of tobacco products and ascertain their known characteristics.
2) Conduct preliminary studies to verify important characteristics of the models—when necessary, develop data on important baseline characteristics of the models; adopt methods to measure tissue toxicity to the models; gain experience working with the models; and finally identify appropriate positive controls for the major toxicity endpoints.
3) Utilize the most applicable models and endpoints to assess the toxicity of a series of cigarette smoke condensates predicted to differ in their toxicity.

PI: Howard, Paul C., Ph.D.

CTP Prep Studies on Inhalation Toxicity (P00753)

Responsible Office: Office of Scientific Coordination
Collaborating Division: Biochemical Toxicology
Collaborating FDA Center: CTP
Objectives:
1) Conduct literature review on inhalation toxicology studies conducted with NNK
2) Conduct literature review on pharmacokinetic studies of NNK following any exposure route.
3) Determine the specifications for an inhalation unit.

PI: Khan, Saeed A., Ph.D.

Gene-Expression Responses by Avirulent *Bacillus Anthracis* and Human Epithelial Cells (EC) During Initial Host-Pathogen Contact (E0733401)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination

Collaborating FDA Center/Office: CDER, ORA

Objectives:

1) Compare the gene-expression profiles of the bacteria and cell lines with and without co-culture.
2) Analyze data for tissue-related differences related to pathogenesis.
3) Validate gene-expression data by RT-qPCR.
4) Identify key signal-transduction pathways and immune-system interaction genes involved in EC stimulation by *B. Anthracis.*

PI: Leakey, Julian E., Ph.D.

Studies of Usnic Acid and *Usnea Barbata* Herb in Rats and Mice (E0215911)

Responsible Office: Office of Scientific Coordination

Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office Center Director/OR

Collaborating FDA Center: CFSAN

Objective:

Establish doses of usnic acid and *Usnea Barbata* preparations, administered in feed, in male and female Fischer 344 rats and B6C3f1 mice, for use in subsequent studies.

PI: Leakey, Julian E., Ph.D.

Subchronic Studies of Usnic Acid in Rats and Mice (E0216501)

Responsible Office: Office of Scientific Coordination

Collaborating Divisions/Office: Biochemical Toxicology, Personalized Nutrition and Medicine, Systems Biology, Office of the Center Director/OR

Objective:

Evaluate the subchronic toxicity of usnic acid in male and female Fischer 344 rats and B6C3F1 mice.

PI: Leakey, Julian E., Ph.D.

Subchronic Toxicology Studies of *Usnea* Lichen in Rats and Mice (E0216601)

Responsible Office: Office of Scientific Coordination

Collaborating Divisions/Office: Biochemical Toxicology, Personalized Nutrition and Medicine, Systems Biology, Office of the Center Director/OR

Objective:

Evaluate the sub-chronic hepatotoxicity of *Usnea* lichen in male and female Fischer 344 rats and B6C3F mice.

PI: Matson, Sandra, Ph.D.

Chemical and Purity Analyses of Glucosamine Sulfate and Salts (P00750)

Responsible Office: Office of Scientific Coordination

Collaborating Division: Biochemical Toxicology

Objectives:

1) Provide chemistry data in support of future glucosamine animal studies.
2) Identify a supply of high-quality glucosamine sulfate, glucosamine sulfate potassium chloride and glucosamine sulfate sodium chloride for subsequent protocols.

PI: Melchior, William B., Ph.D.

Real-Time PCR Assays for Ricin and Related Potential Bioterrorism Agents in Foods (P00684)

Responsible Division: Biochemical Toxicology

Objectives:

1) Develop the precise materials and methods needed to perform the

proposed assays.
2) Prove that the assays work simply, rapidly, and reliably.
3) Prove that the assays function as desired in real-world situations, such as with contaminated food stuffs.

PI: Moore, Martha M., Ph.D.

Evaluation of the Ability of Standard Genetic Toxicology Assays To Assess the Relative Genotoxic Potential of Cigarette Smoke Condensates (E0745901)

Responsible Division: Genetic and Molecular Toxicology
Collaborating FDA Center: CTP
Objectives:
1) Optimize short-term assays to evaluate their ability to initially assess the genotoxicity of cigarette smoke condensates. Based on the results from these studies, further research using commercial cigarettes and whole cigarette smoke will be designed.
2) Attain a dynamic potency range adequate to detect reductions of select harmful and potentially harmful constituents by 30, 50, and 70 percent.
3) Develop and validate a quantitative assay or assays to detect statistically significant differences in cigarette-smoke cytotoxicity over a range of biologically relevant concentrations.
4) Evaluate genotoxicity assays to determine their robustness, sensitivity, reproducibility, and accuracy.

PI: Nawaz, Mohamed S., Ph.D.

Isolation and Characterization of Fluoroquinolone-Resistant Bacteria from Shrimp (E0730701)

Responsible Division: Microbiology
Collaborating Office: Office of the Center Director/OR
Collaborating FDA Center: CVM
Objectives:
1) Isolate and identify fluoroquinolone-resistant Gram-negative bacteria from shrimp imported from different countries.
2) Conduct molecular characterization of fluoroquinolone-resistant determinants.
3) Conduct molecular typing of fluoroquinolone-resistant bacteria.

PI: Nayak, Rajesh R., Ph.D.

Antimicrobial-Resistance Genetics of "Emerging" *Salmonella Enterica* Serovar *Javiana* Phenotypes Involved in Clinical and Food-Related Outbreaks (E0726701)

Responsible Division: Microbiology
Objectives:
1) Determine the intrinsic resistance of *Salmonella Javiana* isolates to multiple antimicrobials by the SensiTitre antimicrobial susceptibility testing protocol using the Clinical and Laboratory Standards Institute guidelines.
2) Determine the variation in genetic clonality among the drug-resistance genotypes by fingerprinting the bacteria using CDC's pulsed-field gel electrophoresis protocol.
3) Identify the genes in the multiple antibiotic region of the *Salmonella* Genomic Island (SGI)-class 1 integron gene cassettes in the resistant phenotypes.
4) Detect antimicrobial-resistance genes in select multi-drug resistant *Javiana* isolates by a PCR-based and microarray biochip methodologies.

PI: Nayak, Rajesh R., Ph.D.

Investigating the Mechanisms of Drug Resistance and Pathogenicity in Clinical *Escherichia Coli* Isolates from Veterinary Sources (E0744901)

Responsible Division: Microbiology
Collaborating FDA Center: CVM
Objectives:

1) Evaluate the antimicrobial susceptibility profiles in *E. coli* and detect the prevalence of antimicrobial resistance genes for the resistant phenotypes.
2) Investigate the mutational changes in gyrase and regulatory genes, and assess the role of plasmids and integrons in mediating the drug resistance.
3) Characterize the molecular basis of drug resistance in *E. coli* displaying extended-spectrum beta-lactamase (ESBL)-phenotypes.
4) Evaluate the virulence gene profiles of the isolates.

PI: Nayak, Rajesh R., Ph.D.

Microbial Genetics of Non-0157:H7 Shiga-Like Toxin Producing *Escherichia Coli* Insulated From Humans and Foods (E0735701)

Responsible Division: Microbiology
Objectives:

1) Obtain *E. coli* isolates from clinical, food-related outbreaks and veterinary diagnostics samples.
2) Map the epidemiological profiles of the isolates for specific genetic markers attributable to the origin of isolates and their phenotypic diversity.
3) Determine the antimicrobial resistance profiles and potential mechanisms of drug resistance among EHECs of various serogroups.
4) Identify the antimicrobial resistance and virulence gene determinants in the bacterial isolates that contribute to their pathogenicity.
5) Examine the role of plasmids, if any, in mitigating the transfer of drug resistance.
6) Compare the cytoxicities of selected EHEC strains to cultured RAW264.7 macrophage cells.
7) Evaluate the expression of Shiga-like toxin using *in vitro* enzyme assays.

PI: Paule, Merle G., Ph.D.

Developmental Neurotoxicity Assessment of Acrylamide in Rats – Long-Term Studies (E0215101)

Responsible Division: Neurotoxicology
Collaborating Divisions: Biochemical Toxicology, Personalized Nutrition and Medicine, Systems Biology
Objective:
Determine the consequences of long-term exposure to acrylamide on a variety of developmental milestones and measures of nervous-system integrity throughout life.

PI: Paule, Merle G., Ph.D.

Due-Diligence for the Development of Rodent and Nonhuman Primate Nicotine Self-Administration Laboratories at NCTR (E0745601)

Responsible Division: Neurotoxicology
Collaborating FDA Center: CTP
Objective:
Define self-administration laboratory needs, including personnel, equipment, space, and maintenance requirements.

PI: Pence, Lisa M., Ph.D.

Development and Optimization of Quantitative LC/MS Metabolic Profiles for Amino Acids, Vitamins, and Other Important Metabolites (P00725)

Responsible Division: Systems Biology
Objective:
Develop and optimize quantitative LC/MS metabolic profiles for markers that have been identified as biomarkers in drug-safety studies such as amino acids, water soluble and fat soluble vitamins, bile acids (biomarkers of liver function), and other metabolites, like gluthathione and SAMe in biofluids.

PI: Rafii, Fatemeh, Ph.D.

Microarray Analysis for the Detection of Targeted Gene-Expression Changes Resulting from Exposure of *Clostridium Perfringens* to Fluoroquinolones (E0731101)

Responsible Division: Microbiology
Collaborating FDA Center: CBER
Objective:
Determine the effect of fluoroquinolone exposure on gene expression and regulation of transcription and metabolic activities of *Clostridium perfringens*.

PI: Stingley, Robin L., Ph.D.

Literature Review of Inhalation Toxicology of 4-(methylnitrosamino)-1-3-pyridyl-1-butanone (E0746401)

Responsible Office: Office of Scientific Coordination
Collaborating FDA Center: CTP
Objectives:
1) Conduct literature review on a) inhalation toxicology studies conducted with NNK and b) pharmacokinetic studies of NNK following any exposure route.
2) Determine the specifications for an inhalation unit and develop plan for installation, including any modification to existing animal space.

PI: Sung, Kidon, Ph.D.

Quantification Proteomic, Transcriptomic, and Phenotypic Microarray Analysis of *C. Jejuni* for the Identification of Colonization Factors in Poultry (E0735601)

Responsible Division: Microbiology
Collaborating Division/Office: Systems Biology, Office of Scientific Coordination
Objectives:
1) Evaluate genomic and phenotypic microarrays, and whole proteomic analyses to compare genes, phenotypes, and proteins from both good and poor *C. Jejuni* chicken colonizers.
2) Investigate functional role of identified colonizing factors by mutant construction, *in vitro* assays, and *in vivo* assays.
3) Identify potential targets for vaccine that will enable us to eliminate the threat of *Campylobacter* infection in chickens.

PI: Sutherland, John B., Ph.D.

Microbial Degradation of Fluoroquinolone Antimicrobial Agents (E0722701)

Responsible Division: Microbiology
Collaborating Division: Biochemical Toxicology
Objective:
Identify microorganisms that either completely degrade fluoroquinolones or modify the fluoroquinolone molecule so as to reduce its toxicity to bacteria.

PI: Sutherland, John B., Ph.D.

Reducing Health Risks from Antimicrobial-Resistant Bacteria by Eliminating Environmental Reservoirs of Resistance (E0738201)

Responsible Division: Microbiology
Collaborating Divisions/Office: Biochemical Toxicology, Systems Biology, Office of the Center Director/OR
Collaborating FDA Center: CVM
Objective:
Identify the specific bacteria and enzymes in the environment that are able to degrade fluoroquinolones to products without antimicrobial activity.

PI: Tolleson, William H., Ph.D.

Chemical Inactivation of Protein Toxins on Food-Contact Surfaces (E0730301)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objectives:

1) Identify cleaning/sanitizing treatments that result in elimination and/or inactivation of protein toxins (abrin and ricin) on food-contact surfaces.
2) Identify surrogate(s) that can be used to study chemical inactivation of abrin or ricin
3) Measure the loss of ricin and abrin biological and biochemical activities in the presence of cleaning/sanitizing solutions using RAW264.7 macrophage cytotoxicity assays and 28S rRNA adenosine N-glycosidase RTqPCR-based enzyme assays.

PI: Tolleson, William H., Ph.D.

Laboratory Studies in Melamine and Cyanuric Acid Biochemical Toxicology (E0729101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Systems Biology
Objective:
Determine chemical and biochemical properties of melamine and cyanuric acid that may influence their toxicity and retention as tissue residues.

PI: Tolleson, William H., Ph.D.

Rapid Detection of Ribosome-Inactivating Protein Toxins in Foods (E0736101)

Responsible Division: Biochemical Toxicology
Collaborating Division: Microbiology
Collaborating FDA Center: CFSAN
Objective:
Provide robust methods for detecting the biological activity of the potential bioterrorism agents ricin, abrin, and shiga-like toxins, each of which is characterized as a ribosome-inactivating protein toxin, in three selected foods (spinach, apple juice, and milk).

PI: Tolleson, William H., Ph.D.

Thermodynamic Measurements for Inactivation of Bioterrorism Agents Ricin and Abrin (P00708)

Responsible Division: Biochemical Toxicology
Collaborating FDA Center: CFSAN
Objectives:

1) Measure forward-rate constants for thermal denaturation of ricin and abrin at seven temperatures (60, 65, 70, 75, 80, 85, 90, and 95 C) and three buffer combinations (0.10 M

NaCl buffered with 20 mM lactate, pH 3.0; 20 mM acetate, pH 5.0; and 20 mM phosphate, pH 7.0) by monitoring the quenching of intrinsic protein (tryptophan) fluorescence (EX295, EM340) in a thermostatted spectrofluorimeter.
2) Exploit results (Tm, Delta H) using differential-scanning calorimetry at NCFST to select Tm for toxin proteins and measure reverse-rate constants (protein renaturation) at one temperature and one buffer combination. Calculate Keq and Delta G from ratio of rates. Determine T Delta S from Delta G and Delta H.
3) Determine the influence of solvent pH on isothermal toxin folding/unfolding equilibria.
4) Identify time-, pH-, and temperature-dependent reversible and irreversible transitions in ricin conformation and correlate these with changes in toxin-dependent enzyme activity and cytotoxicity.

PI: Trbojevich, Raul, Ph.D.

Study of Nanoparticles Migration from Food-Contact Nanomaterials: Characterization and Quantification of Silver Nanoparticles in Stimulants (E0736801)

Responsible Division: Biochemical Toxicology
Collaborating Office: Office of Scientific Coordination
Collaborating FDA Office: ORA
Objectives:
1) Study migration of nanoparticles from nanocomposites used in food-contact materials.
2) Characterize and quantify silver nanoparticles in food simulants.

PI: Wagner, Robert D., Ph.D.

Mechanistic Evaluation of the Induction of Lymphoproliferation and Apoptosis Inhibition by Probiotic Bacteria in Mice Infected with *Salmonella Enterica* (E0727601)

Responsible Division: Microbiology
Objectives:
1) Orally challenge defined human microbiota-associated (HMA) BALB/c mice and probiotic bacteria-treated HMA BALB/c mice with *Salmonella enterica* and isolate intestinal mucosal-associated lymphoid tissues (MALT), including Peyer's patches, lamina propria, and mesenteric lymph nodes.
2) Use pathway-focused gene-expression profiles generated from real-time RT-PCR expression arrays to compare signal transduction in MALT from HMA mice treated with or without probiotic bacteria and orally challenged with *S. enterica*.
3) Develop immunohistochemical (IHC) and *in situ* hybridization (ISH) conditions to detect the expression of the signal pathway molecules implicated in activation and apoptosis inhibition in mucosal T-cells and accessory cells in tissue sections of Peyer's patches, lamina propria, and mesenteric-lymph nodes.
4) Conduct IHC and ISH studies on tissue sections for detection of molecules involved in the regulation of lymphocyte activation and programmed cell-death pathways induced by bacterial-surface antigens.

5) Compare the probiotic-treated and untreated mice for expression of dendritic cell, macrophage, and IEC-derived cytokines.

PI: Wilkes, Jon G., Ph.D.

Rapid Screening of Food or Drugs for Chemical or Microbiological Contamination (E0734701)

Responsible Division: Systems Biology
Objectives:

1) Detect bacterial or chemical contamination in foods or drug ingredients at a concentration of 0.01 to 0.1% by weight in 15 seconds/sample using a pyrolysis mass spectrometry instrumental method combined with pattern recognition.
2) Recognize variant batches, indicate the probable contamination as biological or chemical, and flag suspicious products for analysis.

PI: Word, Beverly R., Ph.D.

DNA Methylation is Modulated by Lifestyle Factors and Environmental Agents (P00713)

Responsible Office: Office of the Center Director/ADRA
Objectives:

1) Determine the effect of cigarette smoke condensate on DNA methylation of several genes in lung cells.
2) Assess the ability of other agents to modulate the effect of CSC (class-specific correlations) on gene DNA methylation, either singularly or in various combinations.

PI: Yang, Xi, Ph.D.

Preliminary Concentration Response Assessments of Tobacco Smoke Condensates in Lung and Cardiac Cells (E0744701)

Responsible Division: Systems Biology
Collaborating Office: Office of the Center Director/ADRA
Collaborating FDA Product Center: CTP
Objective:
Assess the concentration response of three different tobacco smoke condensates (TSC) in two primary lung-cell types, two cardiac-cell types, and two immortal lung-cell types. A battery of endpoints will be used to assess the adverse effects caused by the TSCs at different concentrations and over time. These results will be used to design subsequent omics studies under a separate protocol.

FY 2011 Publications

Publication is an essential component of research. All documents authored by NCTR investigators must undergo the NCTR Document Review and Approval Process, which consists of the review, clearance, and approval by the Center Director prior to submitting the publication to a journal. The list below identifies the NCTR-approved publications that were **accepted or published in journals in FY 2011, and book chapters that were accepted in FY 2011**.

1. Akiyama, T., Khan, A.A. (2012). Molecular characterization of strains of fluoroquinolone-resistant *Salmonella enterica* serovar Schwarzengrund carrying multidrug resistance isolated from imported foods. *Journal of Antimicrobial Chemotherapy*. 67:101-110.
   Responsible Division:Microbiology

2. Akiyama, T., Khan, A.A., Cheng, C., and Stefanova, R. (2011). Molecular characterization of *Salmonella enterica* serovar Saint Paul isolated from imported seafood, pepper, environmental, and clinical samples. *Food Microbiology.* 28:1124-1128.
   Responsible Division:Microbiology

3. Ali, A.A., Lewis, S.M., Yang, X., Salminen, W.F., and Leakey, J.E. (2012). Herbals and dietary nutrients associated with weight loss. *Obesity.* CRC Press. Chapter 52:777-800.
   Responsible Division:Systems Biology
   Co-Author Division:Office of Scientific Coordination

4. Ali, A.A., Salminen, W.F., and Leakey, J.E. (2011). Hexosamine flux and the efficacy and safety of glucosamine in the treatment of osteoarthritis. *Arthritis:Pathophysiology, Prevention, and Therapeutics*. Book Chapter:262-282.
   Responsible Division:Systems Biology
   Co-Author Division:Office of Scientific Coordination

5. Ali, R., Shaddock, J.G., Mittelstaedt, R.A., Ding, W., Bhalli, J.A., Khan, Q., and Heflich, R.H. (2011). Comparative analysis of micronuclei and DNA damage induced by Ochratoxin A in two mammalian cell lines. *Mutation Research*. 723(1):58-64.
   Responsible Division: Genetic and Molecular Toxicology

6. Antony, A.C., Tang, Y., Khan, R.A., Zhang, Y., Xiao, S., Wang, M., Hansen, D.K., and Jayaram, H.N. (2011). Incrimination of human heterogeneous nuclear ribonucleoprotein E1 (hnRNP-E1) as a candidate sensor of physiological folate deficiency. *Journal of Biological Chemistry*. 286(45):39100-15.
Responsible Division:Personalized Nutrition and Medicine

7. Arlt, V.M., Singh, R., Stiborova, M., Gamboa Da Costa, G., Farmer, P.B., Wolf, R.C., Henderson, C.J., and Phillips, D.H. (2011). Effect of hepatic cytochrome P450 (P450) oxidoreductase deficiency on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-DNA adduct formation in P450 reductase inducible conditional null mice. *Drug Metabolism and Disposition.* 39:2169-73.
Responsible Division: Biochemical Toxicology

8. Beger, R. and Colatsky, T. (2012). Metabolomics data and the biomarker qualification process. *Metabolomics*. 8(1):2-7.
Responsible Division:Systems Biology

9. Bhalli, J.A., Pearce, M.G., Dobrovolsky, V.N., and Heflich, R.H. (2011). Manifestation and persistence of Pig-a mutant red blood cells in C57BL/6 mice following single and split doses of N-Ethl-N-Nitrosourea. *Environmental and Molecular Mutagenesis*. 52:9:766-773.
Responsible Division:Genetic and Molecular Toxicology

10. Bhalli, J.A., Shaddock, J.G., Pearce, M.G., Dobrovolsky, V.N., Cao, X., and Heflich, R.H. (2011). Report on stage III Pig-a mutation assays using benzo[a]pyrene. *Environmental and Molecular Mutagenesis.* 52:9:731-737.
Responsible Division:Genetic and Molecular Toxicology

11. Binienda, Z.K., Gough, B.J., Pereira, F., Macedo, T.R., Ali, S.F., and Ribeiro, C.F. (2011). Buprenorphine modulates methamphetamine-induced dopamine dynamics in the rat caudate nucleus,. *Neurotox Res.* 19(1):94-101.
Responsible Division: Neurotoxicology

12. Bisgin, H., Liu, Z., Fang, H., Xu, X., and Tong, W. (2011). Mining FDA drug labels using an unsupervised learning technique–topic modeling. *BMC Bioinformatics.* 12(10):S11.
Responsible Division: Systems Biology

13. Bradford, B.U., Lock, E.F., Kosyk, O., Kim, S., Uehara, T., Harbourt, D., Desimone, M., Threadgill, D., Tryndyak, V.P., Pogribny, I.P., Bleyle, L., Koop, D.R., and Rusyn, I. (2011). Interstrain differences in the liver effects of trichloroethylene in a multistrain panel of inbred mice. *Toxicology Sciences*. 120(1):206-17.
Responsible Division:Biochemical Toxicology

14. Brannen, K., Fenton, S., Hansen, D.K., Harrouk, W., Kim, J., and Shuey, D.L. (2011). Developmental Toxicology - New directions workshop refining testing strategies and study designs. *Birth Effects Research Part B*. 92:404-412.
Responsible Division:Personalized Nutrition and Medicine

15. Camacho, M., Kelly, K.P., Beland, F.A., and Gamboa Da Costa, G., (2011). Gene expression of biomarkers of nephrotoxicity in F344 rats co-exposed to melamine and cyanuric acid for seven days. *Toxicology Letters*. 206:166-171.
Responsible Division:Biochemical Toxicology
Co-Author Division:Office of the Center Director/Office of Research

16. Cammerer, Z., Bhalli, J.A., Cao, X., Coffing, S.L., Dickinson, D., Dobo, K.L., Dobrovolsky, V.N., Engel, M., Fiedler, R.D., Gunther, W.C., Heflich, R.H., Pearce, M.G., Shaddock, J.G., Shutsky, T.J., Thiffeault, C.J., and Schuler, M. (2011). Report on stage III Pig-a mutation assays using N-ethyl-N-nitrosourea comparison with other i*n vivo* genotoxicity endpoints. *Environmental and Molecular Mutagenesis*. 52:9:721-730.
Responsible Division:Genetic and Molecular Toxicology

17. Chae, M., Chen, C., and Chen, J.J. (2011). Reordering hierarchical clustering analysis based on bilateral symmetric distance. *PLoS One*. 6(8):e22546.
Responsible Division:Personalized Nutrition and Medicine

18. Chan, P., Malarkey, D.E., Kissling, G.E., Travlos, G.S., and Fu, P.P. (2011). Two-year toxicity and carcinogenicity studies of panax ginseng in Fisher 344 rats and B6C3F1 mice. *The American Journal of Chinese Medicine.* Vol. 39(4), 779-788.
Responsible Division:Biochemical Toxicology

19. Chang, C., Beland, F.A., Hines, W.M., Fuscoe, J., Han, T., and Chen, J.J. (2011). Identification and categorization of liver toxicity markers induced by a related pair of drugs. *International Journal of Molecular Science*. 12:4609-4624.
Responsible Division:Personalized Nutrition and Medicine
Co-Author Division:Biochemical Toxicology, Systems Biology

20. Chelonis, J.J., Gravelin, C.R., and Paule, M.G. (2011). Assessing motivation in children using a progressive ratio task. *Behavioural Processes.* 87:203-209.
Responsible Division: Neurotoxicology
Co-Author Division: Office of the Director/OR

21. Chen, D., Li, Z., and Chen, T., (2011). Increased expression of miR-34a in mouse spleen one day after exposure to N-ethyl-N-nitrosourea. *Journal of Applied Toxicology*. 5:496-498.
Responsible Division:Genetic and Molecular Toxicology

22. Chen, J.J. (2011). Assessing the performance of differential gene expression analyses. *Pharmacogenomics*. 6:465.
Responsible Division:Personalized Nutrition and Medicine

23. Chen, J.J. (2011). Consistency of predictive signature genes and classifiers. *Pharmacogenomics.* 6:464.
Responsible Division:Personalized Nutrition and Medicine

24. Chen, J.J. and Lin, W. (2011). Identification of preclinical biomarkers of susceptibility to drug-induced toxicity:approach and challenges. *Pharmacogenomics.* 6:493-501.
Responsible Division:Personalized Nutrition and Medicine

25. Chen, M., Vijay, V., Shi, Q., Liu, Z., Fang, H. ,and Tong, W. (2011). FDA-approved drug labeling for the study of drug-induced liver injury. *Drug Discovery Today*. 16(15-16):697-703.
Responsible Division:Systems Biology

26. Chen, M., Shi, L., Kelly, R., Perkins, R.G., Fang, H. and Tong, W. (2011). Selecting a single model or combining multiple models for microarray-based classifier development—A comparative analysis based on large and diverse datasets generated from the MAQC-II project. *BMC Bioinformatics.* 12(10):S3.
Responsible Division: Systems Biology

27. Chen, T., Li, Z., Yan, J., Yang, X., and Salminen, W.F. (2012). MicroRNA expression profiles distinguish the carcinogenic effects of riddelliine in rat liver. *Mutagenesis.* 27:1:59-66.
Responsible Division:Genetic and Molecular Toxicology
Co-Author Division:Systems Biology

28. Chen, X. and Chen, T. (2011). Roles of microRNA in DNA damage and repair. *In Tech.* 18:341-354.
Responsible Division:Genetic and Molecular Toxicology

29. Chen, X., Yan, J., and Chen, T. (2011). Expression level of miR-34a rather than P53 genes status correlates with mutability in related human lymphoblast cell lines. *Molecular Carcinogenesis*.
Responsible Division:Genetic and Molecular Toxicology

30. Dertinger, S.D. and Heflich, R.H. (2011). *In vivo* assessment of Pig-a gene mutation-recent developments and assay validation. *Environmental and Molecular Mutagenesis*. 52:681-684.
Responsible Division:Genetic and Molecular Toxicology

31. Dertinger, S.D., Phonethepswath, S., Weller, P., Nicolette, J., Murray, J., Sonders, P., Vohr, H., Shi, J., Krsmanovic, L., Gleason, C., Custer, L., Henwood, A., Sweder, K., Stankowski, L.F., Roberts, D.J., Giddings, A., Kenny, J., Lynch, A.M., Defrrain, C., Nesslany, F., Van Der Leede, B.M., Doninck, T., Schuermans, A., Tanaka, K., Hiwata, Y., Tajima, O., Wilde, E., Elhajouji, A., Gunther, W.C., Thiffeault, C.J., Shutsky, T.J., Fiedler, R.D., Kimoto, T., BHALLI, J.A., Heflich, R.H., and MacGregor, J.T. (2011). International Pig-a gene mutation assay trial: Evaluation of transferability across fourteen laboratories. *Environmental and Molecular Mutagenesis*. 52(9):690-698.
Responsible Division:Genetic and Molecular Toxicology

32. Ding, W., Levy, D., Bishop, M.E., Lyn-Cook, L.E., Kulkarni, R.M., Chang, C., Aidoo, A., and Manjanatha, M. (2011). Methyleugenol of genotoxicity in the Fisher 344 rat using the Comet Assay and pathway-focused gene expression profiling. *Toxicological Sciences*. 123:1:103-112.
Responsible Division:Genetic and Molecular Toxicology
Co-Author Division:Personalized Nutrition and Medicine

33. Dobrovolsky, V.N., Cao, X., Bhalli, J.A., and Heflich, R.H. (2012). Detection of Pig-a mutant erythrocytes in the peripheral blood of rats and mice. *Molecular Toxicology Protocols*. Book Chapter.
Responsible Division:Genetic and Molecular Toxicology

34. Dobrovolsky, V.N., Mendoza, M., Elespuru, R.K., Bigger, C.H., Robison, T., and Heflich, R.H. (2011). Monitoring humans for somatic mutation in the endogenous Pig-A gene using red blood cells. *Environmental and Molecular Mutagenesis.* 52:9:784-794.
Responsible Division:Genetic and Molecular Toxicology
Co-Author Division:Personalized Nutrition and Medicine

35. Doerge, D.R., Twaddle, N.C., Vanlandingham, M., and Fisher, J.W. (2011). Pharmacokinetics of bisphenol A in neonatal and adult CD-1 mice:Inter-species comparisons with Sprague-Dawley rats and rhesus monkeys. *Toxicology Letters*. 207:298-305.
Responsible Division:Biochemical Toxicology

36. Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Brown, R.C., and Fisher, J.W. (2011). Distribution of Bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats. *Toxicology and Applied Pharmacology*. 255(3):261-70.
Responsible Division:Biochemical Toxicology

37. Doerge, D.R., Vanlandingham, M., Twaddle, N.C., and Delclos, K.B. (2010). Lactational transfer of Bisphenol A in Sprague-Dawley rats. *Toxicology Letters*. 199:372-376.
Responsible Division:Biochemical Toxicology

38. Fakhr, M.K., Noormohamed, A., and Foley, S.L. (2012). Multilocus sequence typing and other sequence-based sub-typing methods to characterize foodborne pathogens. *Molecular Typing Methods for Tracking Foodborne Microorganisms.* Nova Scientific Publishing. Chapter 9:227-254.
Responsible Division:Microbiology

39. Fan, X., Fan, X., Shao, L., Fang, H., Tong, W. and Cheng, Y. (2011). Cross-platform comparison of microarray-based multiple-class prediction. *PLoS ONE.* 6(1):e16067.
Responsible Division: Systems Biology

40. Feng, J., Cerniglia, C.E., and Chen, H. (2012). Toxicological significance of azo dye metabolism by human intestinal microbiota. *Frontiers in Bioscience*. E4:568-586.
Responsible Division:Microbiology

41. Fisher, J.W., Twaddle, N.C., Vanlandingham, M., and Doerge, D.R. (2011). Pharmacokinetic modeling:prediction and evaluation of route dependent dosimetry of Bisphenol A in monkeys with extrapolation to humans. *Toxicology and Applied Pharmacology*. 257(1):122-136.
Responsible Division:Biochemical Toxicology

42. Foley, S.L. (2012). Methods for the analysis of molecular typing data. *Molecular Typing Methods for Tracking Foodborne Microorganisms.* Nova Scientific Publishing. Chapter 14:343-368.
Responsible Division:Microbiology

43. Foley, S.L., Lynne, A.M., Nayak, R.R., Shukla, S.K., and Johnson, T.J. (2012). Subtyping of bacterial foodborne pathogens: Phenotypic methods and an introduction to molecular methods. *Molecular Typing Methods for Tracking Foodborne Microorganisms.* Nova Scientific Publishing. Chapter 6:171-186.
Responsible Division:Microbiology

44. Foley, S.L., Nayak, R.R., Hanning, I., Johnson, T.J., Han, J., and Ricke, S. (2011). Population dynamics of *Salmonella enterica* serotypes in commerical egg and poultry production. *Applied and Environmental Microbiology*. 77(13):4273-4279.
Responsible Division:Microbiology

45. Foley, S.L., Nayak, R.R., Shukla, S.K., and Stemper, M.E. (2012). Other restriction-based typing methods. *Molecular Typing Methods for Tracking Foodborne Microorganisms.* Nova Scientific Publishing. Chapter 8:205-226.
Responsible Division:Microbiology

46. Galloway, S., Lorge, E., Kirkland, D., Aardema, M., Fellows, M., Heflich, R.H., Levy, D., Lynch, A.M., Morita, T., Schuler, M., Speit, G., Eastmond, D. and Marzin, D. (2011). Workshop summary: Top concentration for in vitro mammalian cell genotoxicity assays; and report from working group on toxicity measures and top concentration for in vitro cytogenetics assays (chromosome aberrations and micronucleus), *Mutation Research,* 723:77-83.
Responsible Division: Genetic and Molecular Toxicology

47. Gao, Y., Gopee, N., Howard, P., Walker, N.J., Smith, C.S., and Yu, L. (2011). Proteomic analysis of early responsive lymph node proteins in mice treated with titanium dioxide nanoparticles. *Journal of Proteomics*. 74(12):2745-2759.
Responsible Division:Systems Biology
Co-Author Division:Office of Scientific Coordination

48. Gilbert, M., Mclanahan, E., Hedge, J., Crofton, K., Fisher, J.W., Valentin-Blasini, L., and Blount, B. (2011). Marginal iodide deficiency and thyroid function:Developing an animal model for developmental exposures and a biologically based dose-response model. *Toxicology*. 283:41-48.
Responsible Division:Biochemical Toxicology

49. Gonzalez, C., Salazar-Garcia, S., alestino, G., Martinez-Cuevas, P., Ramirez-Lee, M.A., Jurado-Manzano, B.B., Rosas-Hernandez, H., Gaytan-Pacheco, N., Martel, G., Espinosa-Tanguma, R., Biris, A. and Ali, S.F. (2011). Effect of 45 nm silver nanoparticles (AgNPs) upon the smooth muscle of rat trachea: role of nitric oxide. *Toxicological Letters.* 207:306-313.

50. Guo, L., Dial, S.L., Shi, L., Branham, W.S., Liu, J., Fang, J., Knox, B.L., Deng, H., Kaput, J., and Ning, B. (2011). Similarities and differences in the expression of drug metabolizing enzymes between human hepatic cell lines and primary human hepatocytes. *Drug Metabolism and Disposition*. 39(3):528-538.
Responsible Division:Biochemical Toxicology
Co-Author Division:Genetic and Molecular Toxicology, Personalized Nutrition and Medicine, Systems Biology

51. Han, J., David, D.E., Deck, J., Lynne, A.M., Kaldhone, P., Nayak, R.R., Stefanova, R., and Foley, S.L. (2011). Comparison of *Salmonella enterica* serovar Heidelberg isolates from human patients with those from animal and food sources. *Journal of Clinical Microbiology*. 49(3):1130-1133.
Responsible Division:Microbiology

52. Han, J., Lynne, A.M., David, D.E., Nayak, R.R., and Foley, S.L. (2012). Sequencing of plasmids from a multi-antimicrobial resistance *Salmonella enterica* serovar Dublin strain. *Food Research International*. 45:931-934.
Responsible Division:Microbiology

53. Han, J., Shaheen, B.W., Foley, S.L., and Nayak, R.R. (2012). Prevalence, mechanisms and dissemination of antimicrobial resistance in enteric foodborne bacteria. *Bentham Science Publications, USA*. Chapter 10:151-175.
Responsible Division:Microbiology

54. Han, J., Wang, Y., Shen, Z., Wang, B., Tang, H., Meehan, J.F., Wu, C., and Shen, J. (2012). PCR-based molecular typing methods for tracking foodborne microorganisms. *Molecular Typing Methods for Tracking Foodborne Microorganisms.* Nova Scientific Publishing. Chapter 11:275-300.
Responsible Division:Microbiology

55. Hansen, D.K. and Inselman, A.L. (2011). Applications of stem cells in developmental toxicology. *Reproductive and Developmental Toxicology*. Pgs. 783-792.
Responsible Division:Personalized Nutrition and Medicine

56. Hansen, D.K., Juliar, B., White, G.A., and Pellicore, L. (2011). Developmental toxicity of Citrus aurantium in rats*. Birth Defects Research Part B:Developmental and ReproductiveToxicology*. 92(3):216-223.
Responsible Division:Personalized Nutrition and Medicine

57. He, G., Thorpe, C., Walsh, D., Crow, R., Chen, H., Kumar, S., and Varela, M.F. (2011). EmmdR, a new member of the MATE family of multidrug transporters, extrudes quinolones from *Enterobacter cloacae*. *Archives of Microbiology*. 193:759-765.
Responsible Division:Microbiology

58. He, G., Zhang, C., Crow, R., Thorpe, C., Chen, H., Kumar, S., Tsuchiya, T., and Varela, M.F. (2011). SugE, a new member of the SMR family of transporters, contributes to antimicrobial resistance in *Enterobacter cloacae*. *Antimicrobial Agents and Chemotherapy*. 55(8):3954-3957.
Responsible Division:Microbiology

59. Heflich, R.H., Kimoto, T., Suzuki, K., Miura, D., Kasahara, Y., Dobrovolsky, V.N., and Kobayashi, X. (2011). Manifestation of Pig-a Mutant bone marrow erythroids and peripheral blood erythrocytes in mice treated with N-Ethyl-N-nitrosourea; Direct sequencing of Pig-a cDNA from cells negative for GPI-anchored protein expression. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis*. 723(1):36-42.
Responsible Division:Genetic and Molecular Toxicology

60. Heflich, R.H., Miura, D., Shaddock, J.G., Mittelstaedt, R.A., Dobrovolsky, V.N., Kimoto, T., and Kasahara, Y. (2011). Analysis of mutations in the Pig-a gene of spleen T-cells from N-ethyl-N-nitrosourea-treated Fisher 344 rats. *Environmental and Molecular Mutagenesis*. 52:419-423.
Responsible Division:Genetic and Molecular Toxicology

61. Hong, H., Perkins, R.G., Shi, L., Fang, H., Slavov, S.H., Mendrick, D.L., and Tong, W. Molecular biomarkers for personalized medicine. *Handbook of Personalized Medicine*.
Responsible Division:Systems Biology

62. Hong, H., Xu, L., Mendrick, D.L., and Tong, W. (2011). Genome-wide association studies of Type 2 diabetes: Current status, open challenges, and future perspectives. *OMICS:Biomedical Perspectives and Applications*. 401-429.
Responsible Division:Systems Biology

63. Hsu, C., Hsu, S., Chen, H., Tseng, T., Liu, C., Niu, W., Jeng, J., Liu, C., Lai, M., Chen, P., Kao, J., and Chen, D. (2011). Association of IL28B gene variations with mathematical modeling of viral kinetics in chronic Hepatitis C Paptients with pegylated interferon plus ribavirin therapy. *Proceedings of the National Academy of Sciences.* 108(9):3719-3724.

64. Inselman, A.L., Hansen, D.K., Lee, H., Nakamura, N., Ning, B., Monteiro, J.P., Varma, V., and Kaput, J. (2011). Assessment of research models for testing gene-environment interactions. *European Journal of Pharmacology*. 668:S108-S116.
Responsible Division:Personalized Nutrition and Medicine

65. Jacob, C. and Gamboa Da Costa, G. (2011). Low-level quantification of melamine and cyanuric acid in limited samples of rat serum by UPLC- electrospray tandem mass spectrometry. *Journal of Chromatography B*. 879(9-10):652-6.
Responsible Division:Biochemical Toxicology

66. Jacob, C., Reimschuessel, R., Von Tungeln, L.S., Olson, G.R., Warbritton, A.R., Hattan, D.G., Beland, F.A., and Gamboa Da Costa, G. (2010). Dose response assessment of the nephrotoxicity of a seven-day combined-exposure to melamine and cyanuric acid in F344 rats. *Toxicological Sciences*.
Responsible Division:Biochemical Toxicology

67. Jeannot, E., Boorman, G.A., Kosyk, O., Bradford, B.U., Shymoniak, S., Tumurbaatar, B., Weinman, S.A., Melnyk, S.B., Tryndyak, V.P., Pogribny, I.P., and Rusyn, I. (2012). Increased incidence of aflatoxin B1-induced liver tumors in Hepatitis Virus C transgenic mice. *International Journal of Cancer*. 130(6):1347-1356.
Responsible Division:Biochemical Toxicology

68. Jeannot, E., Pogribny, I.P., Beland, F.A., and Rusyn, I. (2011). Chronic administration of ethanol leads to an increased incidence of hepatocellular adenoma by promoting H-ras-mutated cells. *Cancer Letters*. 301(2):161-7.
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